Abstracts for Plenary Session Talks

Plenary Session I, Genome Evolution and Dynamics

Evolution of Microsporidia

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The Microsporidia are a diverse group, with over 1,200 described species, composed entirely of highly adapted, obligate intracellular parasites. All growth and division is intracellular, and outside the host cell they only exist as spores. Spores are resistant, largely dormant cells dominated by a complex infection apparatus. Spores are otherwise quite reduced, having lost or severely reduced canonical structures such as mitochondria, peroxisomes, Golgi dictyostomes, or any 9 + 2 microtubular structures. Microsporidia are also reduced at most other levels, having little metabolic diversity and among the smallest nuclear genomes known (as small as 2.9 Mbp). Their genomes are highly reduced (few genes) and compacted (high gene density), and their genes highly divergent, characteristics which have together led to some unusual developments in genome dynamics and function. The extreme simplicity of microsporidian cells was once thought to reflect their ancient, primitive nature, which was originally supported by molecular phylogeny. However, for over a decade now evidence has accumulated that they are in fact related to fungi, a conclusion now strongly supported. Whether microsporidia actually are fungi as opposed to being a sister group to fungi as a whole remained unclear for some time, but both phylogenetic reconstruction and more recently analyses of genome order conservation now both suggest they emerge from within the fungi, probably closely related to zygomycetes. The realization that microsporidia evolved from fungi transforms the way their unusual biology is interpreted - they are no longer considered primitive, and are instead seen to be highly derived.

Host-pathogen interaction drives genome plasticity in the late blight pathogen

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Phytophthora infestans causes late blight of potato and was the proximal cause of the Great Irish Famine. It remains a devastating agricultural problem as it rapidly circumvents efforts at control by use of resistant cultivars and fungicides. The ~240 Mb genome of *P. infestans* is the largest and most complex in the chromalveolate lineage, and its sequence reveals features that illuminate its success as a pathogen. Comparison to other *Phytophthora* genomes shows massive expansion and a high rate of turnover in the content of genes encoding secreted proteins, including the host-translocated effectors of the RXLR class. *P. infestans* also has a vastly expanded array of Crinkler family genes. New functional analyses show that several classes of Crinklers trigger cell death when expressed inside plant cells, suggesting that they form a new class of host-translocated effectors.

The genome structure itself likely plays a role in successful adaptation. The genome includes large repeat-rich regions where genes and gene order are not conserved. These regions are significantly enriched in rapidly evolving genes encoding secreted proteins such as RXLRs and Crinklers, and may provide a mechanism for easily duplicating successful effectors and deleting those that trigger resistance. For example, the Avr1 avirulence gene occurs at duplicated loci, and its avirulence allele is entirely absent in virulent strains. Sample sequencing of several strains of *P. infestans* and closely related *Phytophthora* species shows extensive copy number variation in the majority of secreted effectors, suggesting this as a common mechanism for evading resistance.

The genome of Malassezia globosa, a fungus associated with dandruff

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We have sequenced the genome of *Malassezia globosa* and part of the genome of *Malassezia restricta*, two fungi found on the scalp and associated with dandruff. The *M. globosa* genome is among the smallest genomes of free-living fungi. The Malassezia are most closely related to plant pathogens such as *Ustilago maydis.* However, the set of secreted enzymes (lipases, aspartyl proteases, phospholipases, acid sphingomyelinases) is more similar to the distantly related *Candida albicans* that can also occupy a niche on animal skin. The set of secreted enzymes may reflect an adaptation to animal skin. The *M. globosa* genome reveals evidence of a mating type locus, although mating has not been observed in Malassezia

Horizontal gene transfer and the spread of trophic mechanisms in the eukaryotes

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Horizontal gene transfer (HGT) involves the transmission of genetic material between distinct evolutionary lineages. Large-scale HGT was an important process in the origin of the mitochondrial and chloroplast organelles. Reports of HGT between eukaryotes are, however, far less common and the significance of this process is poorly understood.

Analysis of protozoan and algal genomes suggests incidences of HGT may have been driven by phagotrophic lifestyles and that this has been an important factor in the spread of phototrophy. Ecological association between donor and recipient lineages seems also to be a pre-requisite for HGT, suggesting that plants and fungi, which share numerous habitats, may have also shared genetic information. We tested this idea by carrying out phylogenomic analysis of plant and fungal genomes. This demonstrated a pattern of infrequent gene transfer between these two non-phagotrophic eukaryotic kingdoms, transmitting several distinct metabolic traits. The pattern of HGT implies that the absence of phagotrophy is not a barrier to transmission, but may impede the frequency of transfer. In contrast, our analyses of oomycetes show a number of fungal derived HGTs. The putative function of these transferred genes suggests that these acquisitions were important in the evolutionary history of the oomycetes, which has encompassed a radical change in lifestyle, from ancestral phagotrophic algae to filamentous osmotrophic pathogens.

The origin of plant pathogens: mechanisms of genome evolution during host specialization

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We used the wheat pathogen *Mycosphaerella graminicola* as a model to study genome evolution of closely related pathogens that have specialized on different hosts. *M. graminicola* emerged simultaneously with the domestication of wheat 10-11,000 years ago in the Fertile Crescent through a host jump from wild grasses onto cultivated wheat. The pathogen has since co-evolved and spread with its wheat host, while the “ancestral” species still infect wild grasses in the Middle East.

Our project uses massive parallel sequencing and bioinformatics tools to sequence, assemble and analyse 12 genomes of *Mycosphaerella* sampled from different grass hosts including wheat, barley and wild grasses collected in the Fertile Crescent. This collection of strains will allow us to compare genomes of pathogens that have diverged from their most recent common ancestor over different time frames and in different environments (agro-ecosystem vs. natural vegetation), providing a unique opportunity to study key evolutionary events during host specialization and speciation.

Our initial genome comparison includes the already sequenced *M. graminicola* genome and an isolate from wild grasses named S1. For the 71% of the genomes that can be aligned with confidence, the two sequences show a surprisingly low level of nucleotide divergence (6-8%). This applies to both the nuclear and the mitochondrial genomes. The mating type locus is highly conserved suggesting that diversifying selection in mating type genes was not involved in the sympatric pathogen divergence. Divergence may have been driven by other factors related to host specialization, including the loss or acquisition of particular host-related genes. Our data show that large DNA fragments are unique to each strain, suggesting that large-scale rearrangements may have made a significant contribution to the diversification of *Mycosphaerella* species on different hosts.

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Plenary Session II, Gene Regulation and Metabolism

Regulation and consequences of a fungal antiviral RNA silencing response

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RNA silencing, a highly conserved, RNA-mediated, sequence specific recognition and degradation pathway, plays a key antivirus defense role in plants and influences virus replication in animals. The chestnut blight fungus *Cryphonectria parasitica* and associated mycovirus has served as an excellent experimental system to elucidate the role of RNA silencing as an antiviral defense mechanism in fungi. Disruption of one of two *C. parasitica* dicer genes, *dcl-2*, was found to increase susceptibility to mycovirus infection. Subsequent studies showed that *dcl-2* functions to process mycovirus RNAs into virus-derived small interfering RNAs (vsRNAs) as part of an inducible RNA silencing antiviral response that is sensitive to a mycovirus-encoded suppressor of RNA silencing. The recent release of the *C. parasitica* draft genome sequence by the Joint Genome Institute has greatly facilitated the identification of genes encoding other components of the RNA silencing pathway and characterization of their regulation and role in the antiviral defense response.
Additional studies on *dcl-2* function revealed an unexpected role for this host dicer in viral RNA recombination. This finding has broad implications due to the importance of virus RNA recombination as a component of virus evolution that contributes to the emergence of new viruses and as an obstacles for use of recombinant RNA viruses as gene expression vectors for a variety of practical applications, including gene therapy.

Natural genetic variation and the transcriptome

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Most sequence variants between genetically diverse individuals in a species give rise to subtle regulatory changes rather than to qualitative gains or losses. How these changes propagate through the regulatory network and manifest phenotypically is not well understood. Compact fungal genomes provide an excellent testbed for dissection of the principles of regulatory variation. Here we use a cross between two isolates of *S. cerevisiae* to identify genetic variants that impact expression of mRNAs on a high-throughput scale. We show that a number of sequence variants in coding regions influence expression of their own gene through feedback, and we find evidence for extensive regulatory feedback among yeast DNA-binding proteins. We also show that genetic differences in expression of un-annotated, intergenic transcripts can be harnessed to infer function for these novel RNAs. We extend these paradigms to a comparison between *N. crassa* strains, showing that natural genetic variation in gene expression is widespread in this organism; we also use RNA-seq profiling of *N. crassa* to pioneer a novel method to extract genotyping information from expression measurements.

Metabolism of *Aspergilli* at the genome level

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Aspergilli are used extensively in the fermentation industry for the production of a range of different industrial enzymes, organic acids and high-value secondary metabolites. In connection with further development of bioprocesses for the production of fuels and chemicals these fungi are interesting versatile cell factories as they tolerates low pH, can utilize a wide range of carbon sources and has relatively high conversion rates. Furthermore, their metabolism extends to cover many different secondary metabolites. Due to these factors the metabolism of *Aspergilli* is quite complex and involves a very large number of enzyme catalyzed reactions, probably among the microorganisms containing the largest number of metabolic capabilities. With the availability of the genome sequence for several different *Aspergilli* it has become possible to query the metabolic capabilities of these microorganisms at the genome-level. Using a bottom-up approach using genomic information together with information from databases, research papers and books, we have reconstructed the metabolic networks of three *Aspergilli*: *A. nidulans*, *A. niger* and *A. oryzae*. In connection with the metabolic network reconstruction process we further improved the genome annotation. The metabolic networks can be used as scaffolds for identification of co-regulatory modules in response to different perturbations, e.g. changes in the carbon source and this has allowed us to analyze differences in the metabolic responses between the three species. The metabolic networks can also be converted into so-called genome-scale metabolic models that can be used for identification of metabolic engineering targets. To further analyze how the metabolism is regulated in *Aspergilli* we have used comparative transcriptome analysis of two or three species, using a custom-designed Affymetrix DNA array covering probes for all three species. Through analysis of the promoter sequences of orthologous genes having the same transcriptional response to a metabolic perturbation we could identify consensus binding sites for conserved transcription factors, e.g. for regulation of xylose and glycerol metabolism. We further performed a comparative genomic analysis of *A. niger* and *A. oryzae* with the objective to identify common transcription factors involved in regulation of metabolism, and using transcriptome data from different carbon sources we could map out draft regulatory structures in these fungi. Based on these system biology studies of *Aspergilli* we have started to reconstruct complete metabolic networks and regulatory structures in *A. niger* and *A. oryzae*, which may open up for a wider application of these fungi as cell factories for sustainable production of fuels and chemicals.

Evolution of a secondary metabolite biosynthetic gene cluster in *Fusarium*

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Trichothecenes are mycotoxins produced by some plant pathogenic species of *Fusarium* and can contribute to virulence on some plants. In *Fusarium graminearum* and *F. sporotrichioides* trichothecene biosynthetic enzymes are encoded at three loci: the single-gene *TRI101* locus; the two-gene *TRI1*/*TRI16* locus; and the 12-gene core *TRI* cluster. Here, sequence analysis revealed that *TRI1* and *TRI101*, but not *TRI16*, are located in the core cluster in *F. equiseti*. Examination of genome sequences of distantly related, trichothecene-nonproducing species of *Fusarium* revealed remnants of *TRI101* in the same genetic environment as the intact *TRI101* in *F. graminearum* and *F. sporotrichioides*. This suggests that *TRI101* was present at this location, rather than in the core cluster, prior to divergence of trichothecene-producing and nonproducing species. Additional analyses of 16 trichothecene-producing fusaria revealed that species phylogenies inferred from sequences of primary metabolic and core *TRI* cluster genes were correlated with locations of *TRI1* in the species examined but not with phylogenies inferred from *TRI1* sequences. In addition, phylogenies inferred from *TRI16* sequences were correlated with those of *TRI1* regardless of whether the two genes were at the same locus. This pattern of congruent and incongruent phylogenies suggests that *TRI1* was located near *TRI16* but outside the core *TRI* cluster in the ancestral *Fusarium* and that *TRI1* moved into the cluster during the evolution of *F. equiseti* and related species. Thus, our results provide evidence that a filamentous fungal gene cluster can expand by relocation of genes into the cluster from elsewhere in the same genome.

Control of DNA methylation in Neurospora

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Detailed analyses of the distribution of DNA methylation in the *Neurospora crassa* genome confirmed that it is almost exclusively in relics of RIP. These are mostly in the centromeric regions but are also found near telomeres and at scores of dispersed sites. Our genetic and biochemical studies have revealed clear ties between DNA methylation and chromatin modifications. In vegetative cells, the DIM-2 DNA methyltransferase is directed by heterochromatin protein 1 (HP1), which in turn recognizes trimethyl-lysine 9 on histone H3, placed by the DIM-5 histone H3 methyltransferase. DIM-5 is sensitive to modifications of histones including methylation of Lysine 4 and phosphorylation of Serine 10. We purified epitope-tagged DIM-5 and found in to be in a complex with four other proteins that are also essential for DNA methylation. We have also identified several proteins that control the extent of DNA methylation. For example, mutants in *mdm-1* (modulator of DNA methylation -1) or *mdm-2* (encodes protein partner of MDM-1) show aberrant methylation of DNA and histone H3K9, with both frequently spreading into genes adjacent to transposable elements. Mutants defective in *mdm-1* mutants grow poorly but growth can be restored by reduction or elimination of DNA methylation using the drug 5-azacytosine or by mutation of the DNA methyltransferase gene, *dim*-2. Mutants defective in both *mdm-1* and *dim-2* display normal H3K9me3 patterns, implying that the spread of H3K9me3 involves DNA methylation. MDM-1 is preferentially localized to edges of methylated regions in an HP1-dependent manner. I will discuss our recent progress towards the elucidation of mechanisms controlling DNA methylation in Neurospora.

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Plenary Session III, Signaling, Development and Sex

mRNA trafficking during pathogenic development of *Ustilago maydis*

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Cytoskeletal transport promotes polar growth in filamentous fungi. In *Ustilago maydis*, the RNA-binding protein Rrm4 shuttles along microtubules and is crucial for establishing the polarity axis in infectious filaments. Mutations in the RNA-binding domain cause loss of function. However, it is unclear which RNAs are bound and transported. Thus, we applied *in vivo* RNA binding studies and RNA live imaging to determine the molecular function of Rrm4. This new combination revealed that Rrm4 mediates microtubule-dependent transport of distinct mRNAs encoding e.g. the ubiquitin fusion protein Ubi1 and the small G protein Rho3. These transcripts accumulate in ribonucleoprotein particles (mRNPs) that move bidirectionally along microtubules and co-localise with Rrm4. Importantly, the 3' UTR of *ubi1* containing a CA-rich binding site functions as zipcode during mRNA transport. Loss of Rrm4 leads to a drastic reduction of motile mRNPs and defects in polar growth suggesting that mRNP trafficking is required for polarity. This is the first example of microtubule-dependent mRNA transport in fungi and our data provide evidence that fundamental principles are conserved among fungi, plants, and animals.

Sex and light signaling in the zygomycete *Phycomyces blakesleeanus.*

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Phycomyces blakesleeanus is a member of the subphylum Mucormycotina that was formerly part of the zygomycetes. As a species of an early diverging lineage in the kingdom it represents a model for understanding the evolutionary processes in fungi. Research on *Phycomyces* has focused on understanding the interactions between light-sensing, the development of sexual and asexual structures, and carotene biosynthesis. Light induces the production of 10 cm high sporangiophores for asexual sporulation, and these structures are phototropic being sensitive to blue and UV wavelengths. Light represses mating, yet also induces carotene biosynthesis. Production of carotene is essential for mating because beta-carotene is the precursor for the trisporic acid pheromones used by *Phycomyces* and related species. Starting more than 30 years ago, chemical mutagenesis and large scale screens resulted in the isolation of strains impaired in phototropism, mating, and carotenogenesis; however, few genes have been identified that correspond to those mutated. The available genome sequence for *Phycomyces* coupled with traditional genetic mapping has now enabled the identification of genes affected in *mad* mutants, encoded by the *sex* (mating type) locus, and required for the biosynthesis of trisporic acid pheromones. Genetic mapping promises to reveal the nature of other mutated genes and thereby the regulatory processes controlling development in *Phycomyces* and the basal fungi.

Regulatory pathways involved in spore formation in the oomycete *Phytophthora infestans*.

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Phytophthora infestans is an important plant pathogen (potato late blight) and a model for studies of growth and development in oomycetes. Most oomycete life cycles include the production and germination of spores, including zoospores, that are critical to their dissemination and pathogenicity. We are applying both molecular genetic, genomic, and proteomic approaches towards understanding the biology of *P. infestans* spores. For example, microarrays representing the transcriptome were used to study early and late stages of sporulation, zoosporogenesis, cyst germination, and plant infection, as well as sexual sporulation. Massive changes were observed between each stage, with more than 1200 transcripts being entirely specific to spores. Functional and bioinformatics-based analyses of their promoters revealed transcription factor binding sites regulating stage-specific expression; studies of the cognate factors should ultimately reveal what regulates processes such as sporulation and germination. Using gene silencing, several genes were shown to play important roles in the spore cycle, such as *Cdc14*. While known in other species as a constitutively expressed regulator of mitosis and the cell cycle, in oomycetes *Cdc14* is only expressed in spores and appears to have evolved a novel function related to sporulation and flagella function. Models for the gain and loss of flagella and associated proteins during evolution will be discussed.

Role of Actin Cytoskeletal Dynamics in Activation of the cyclic AMP signalling Pathway and *HWP1* Gene Expression in *Candida albicans*

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Changes in gene expression during reversible bud-hypha transitions of the opportunistic fungal pathogen *Candida albicans* permit adaptation to environmental conditions that are critical for proliferation in host tissues. Our previous work showed that the hypha-specific adhesin gene, *HWP1*, is upregulated by the cAMP signaling pathway. However, little is known about the potential influences of determinants of cell morphology on *HWP1* gene expression. We found that blocking hypha formation with cytochalasin A, which destabilizes actin filaments, and with latrunculin A, which sequesters actin monomers, led to a loss of *HWP1* promoter activity. In contrast, high levels of *HWP1* gene expresion were observed when the F-actin stabilizer jasplakinolide was used to block hypha formation, suggesting that *HWP1* gene expression could be regulated by actin structures. Mutants defective in formin-mediated nucleation of F-actin were reduced in *HWP1* gene expression, providing genetic support for the importance of actin structures. Kinetic experiments with wild-type and actin-deficient cells revealed two distinct phases of *HWP1* gene expression; a slow, actin-independent phase preceding a fast, actin-dependent phase. Low levels of *HWP1* gene expression that appeared to be independent of stabilized actin and cAMP signaling were detected using indirect immunofluorescence. A connection between actin structures and the activation of the cAMP signaling pathway was shown using hyper- and hypomorphic cAMP mutants, suggesting a possible mechanism for activation of *HWP1* gene expression by stabilized actin. The results reveal a new role for F-actin as a regulatory agent of hypha-specific gene expression at the bud-hypha transition.

ROS signaling and control of fungal development.

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We are interested in the mechanisms that regulate the production, perception and elimination of reactive oxygen species (ROS) and the role of ROS in regulation of fungal development. Enzymes of the NADPH oxidase (NOX) family produce ROS in a regulated manner. A comparative analysis of NOX function in *Aspergillus nidulans* and *Neurospora crassa* shows common and specific functions in both fungi, and implicates ROS in regulation of sexual and asexual development and regulation of polar growth. With regards to ROS perception, we characterized *A. nidulans* response regulator genes *srrA* and *sskA*, as part of a phosphorelay system involved in general stress sensing, as well as other components of the MAP kinase SakA pathway. We found that genes encoding putative transcription factors SrrA, AtfA and NapA are all involved in ROS detoxification and in different aspects of development. Our results support the role of ROS as critical cell differentiation signals.

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Plenary Session IV, Pathogenic and Symbiotic Interactions

Immune recognition of the fungal cell wall.

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The core components of the cell wall are shared by most fungal species and consequently the immune system has evolved to try to detect these molecules to induce protective responses. In the human pathogen *Candida albicans* the cell wall and septal cross-walls are composed of a robust chitin-glucan inner skeleton to which an outer shield of a matrix of highly glycosylated mannoproteins at attached. Emerging evidence that has shown that all of the main components of the *C. albicans* cell wall skeleton and matrix may be recognised, both singly and in combination, by the immune system. However, while some components stimulate immune recognition others attenuate or block it. The composition of the cell wall is also far from fixed, and is modulated during cellular morphogenesis and in response to environmental changes. Therefore the dynamic nature of the cell wall makes it a moving target for the immune surveillance. This presentation will summarise how the fusion of fungal molecular genetics and immunology has led to the systematic dissection of the mechanisms by which cells of the innate immune system grapple and destroy fungal invaders and reciprocally how pathogenic fungi have adapted their cell wall structure create a defensive shield and camouflage jacket to protect themselves from the immunological sentinels of the body.

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Life inside a rice cell; lessons from the blast fungus

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To cause rice blast disease, *Magnaporthe oryzae* sequentially invades living rice cells using specialized invasive hyphae (IH) that exhibit pseudohyphal growth. We are using optically clear leaf sheath tissue to observe pathogen colonization of living rice cells. After fungal appressoria puncture the plant surface, the fungus grows in the host cell lumen as thin filamentous primary hyphae. At successful infection sites, primary hyphae differentiate into IH, which are sealed inside host-derived Extra-Invasive-Hyphal Membrane (EIHM). IH fill first-invaded rice cells, search for locations to cross the plant cell wall, and then send highly constricted IH-pegs across the wall to move into neighboring cells. Our data suggest that IH recognize and manipulate plasmodesmata for their cell-to-cell movement. We are working to identify and characterize blast effector proteins that are secreted into live plant cells to control host defenses and cellular processes. Analysis of IH that secrete fluorescent fusions of blast effectors AVR-Pita, PWL1 and PWL2 identified a novel pathogen-induced structure, the Biotrophic Interfacial Complex (BIC). BICs first appear within the EIHM at primary hyphal tips, and then they move beside the first differentiated IH cell. Fluorescent effector proteins accumulate in BICs as IH continue to grow in the plant cell. In order to identify additional candidate effectors, we optimized the rice sheath assay to obtain infected tissue RNAs with ~20% IH RNA. Microarray analyses using these RNAs identified Biotrophy-Associated Secreted (BAS) proteins that co-accumulate with effectors in BICs, and other BAS proteins that accumulate elsewhere. Mechanisms by which the fungus targets certain secreted proteins to BICs and the potential function for BICs in secretion of blast effectors across the EIHM into the rice cytoplasm will be discussed.

Toxins for life and death, a mechanism of pathogenesis.

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Toxins are produced by a wide array of microbial pathogens and are typically thought to contribute to virulence by promoting host cell death. The presumed primary role of toxins in promoting host cell death may belie the diversity of their functions and even the manner by which they contribute to virulence. We have been working on the toxins produced by the wheat pathogen, *Pyrenophora tritici-repentis*, with the long-term goal being a complete molecular description of this host-pathogen interaction. This includes the identification and characterization of genes involved in pathogenicity and host specificity, the mechanisms by which this fungus acquires these virulence factors, and the determination of the molecular site- and mode-of-action of these toxins. ToxA and ToxB are two proteinaceous HSTs of *P. tritici-repentis* that induce necrosis and chlorosis, respectively. Internalization of ToxA requires the presence of a solvent-exposed, RGD-containing loop on ToxA and a high affinity receptor on toxin-sensitive wheat cells. Internal expression of ToxA reveals a conserved site-of-action in monocots and dicots. Once internalized, ToxA localizes to chloroplasts and is thought to interact with a chloroplast-localized protein, ToxABP1. ToxA induces changes in Photosystem I and II homeostasis and the accumulation of reactive oxygen species. ToxA also induces major transcriptional reprogramming including cellular responses typically associated with defense, suggesting that ToxA acts as both an elicitor and a virulence factor. Site-of-action and structural components necessary for ToxB activity appear to be quite different from ToxA.

Cellular and molecular plant responses to AM fungi

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Arbuscular mycorrhizal (AM) fungi contribute significantly to soil nutrient uptake in plants, but as obligate biotrophs, they depend on their hosts for carbohydrates. Here, we describe how important cellular and transcriptional reprogramming takes place in host cells in the presence of AM fungi during all the steps of the interaction. Plant cell cultures and roots perceive diffusible fungal signals which elicit short and long term responses, as well as transcriptional changes, detected by a Lotus japonicus Affimetrix GeneChip. The physical contact between the AM fungus and the root surface causes a deep reorganization inside the epidermal cells prior to fungal colonization: in vivo confocal imaging studies have shown the formation of cytoplasmic assemblies consisting of cytoskeleton, endoplasmic reticulum, organelles and membrane vesicles, named the prepenetration apparatus. Such events are mirrored by some transcriptional changes, differently from the wide transcriptional reprogramming observed when the fungus colonizes the cortex, and differentiates the arbuscules, which are the key element for the symbiotic nutrient exchange. Here, substantial changes in transcripts involved in regulatory networks, transport, cell wall and membrane biogenesis were detected and located in the arbusculated cells, mirroring parallel confocal microscopy and ultrastructural observations in Medicago and carrot roots. Based on our results, we propose that plant cells respond differently to diffusible signals from AM fungi and to their physical contact, in both cases exerting a direct control on fungal colonization.

Insects in the lives of fungi.

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Fungi are associated with a variety of insects in their environment. Such associations include virulent pathogens, ectoparasitics, yeast-like mutualists, scale insects and beetles that use basidiomata as dwellings, and yeast-like fungi and yeasts from the gut of many insects. The yeasts from the gut of fungus-feeding beetles are of special interest because about 200 undescribed taxa have been isolated from them; 30% of these yeasts belong to the *Candida tanzawaensis* clade, and they appear to have some specificity for this environment, although we do not yet know how they interact with their hosts. Gut yeast physiological profiles are correlated with insect nutritional modes, and as expected the enzymes of mushroom-feeding beetles differ for example from those of wood-consuming beetles. Distribution patterns indicate that many members of the *C. tanzawaensis* clade are isolated both by geography and insect host. Furthermore, many of the yeasts exhibit killer phenomenon that could be an additional isolating mechanism in the restricted environment, perhaps involved in the high species diversity observed among the beetle gut organisms.

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Abstracts for Concurrent Session Talks

CONCURRENT SESSION I

Interactions between fungal pathogens and their human hosts: Ted White and Judith Rhodes

Evolutionary genomics identifies proteins important to the interaction between Coccidioides and its host.
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While most Ascomycetes tend to associate principally with plants, the dimorphic fungi *Coccidioides immitis* and *C. posadasii* are primary pathogens of immunocompetent mammals, including humans. Infection results from environmental exposure to *Coccidiodies*, which is believed to grow as a soil saprophyte in arid deserts. The wealth of Ascomycete genome sequences, which includes the recent addition of 14 strains of *Coccidioides* as well as the Onygenalean relatives *Uncinocarpus reesii* and *Histoplasma capsulatum*, enabled a comparative genomic analysis of *Coccidiodies* at various evolutionary distances. Broad-based comparisons across the filamentous Ascomycota identified changes i n gene family size associated with an evolutionary shift from growth on plants to growth on mammals. A second level analysis among only the Onygenales genomes revealed gene gains and losses and rapidly evolving genes that may have contributed to the evolution of virulence in *Coccidioides*. Finally, population level comparisons among the *Coccidiodies* strains identified secreted and extracellularly localizing proteins e volving under position selection, suggesting that they interact with the host immune system. Overall, the results indicate that *Coccidioides* species are not soil saprophytes, but that they have evolved to remain associated with their dead animal hosts in soil. In addition, the data intimate that *Coccidioides* metabolism genes, membrane related proteins and putatively antigenic compounds have evolved in response to interaction with an animal host.

A sterol-regulatory element binding protein is required for cell polarity, hypoxia adaptation, azole drug resistance, and virulence in *Aspergillus fumigatus*.
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Aspergillus fumigatus is a saprophytic fungus commonly found in soil and compost piles. In immunocompromised patients it takes on a sinister form as a potentially lethal opportunistic human pathogen. At the site of infection, the significant influx of immune effector cells and the necrosis of tissue by the invading pathogen generates a hypoxic microenvironment in which both the pathogen and host cells must survive. Currently, whether hypoxia adaptation is an important virulence attribute is unknown. Here we report the characterization of a sterol-regulatory element binding protein, SrbA, in *A. fumigatus*. Loss of SrbA results in a mutant strain of the fungus that is incapable of growth in a hypoxic environment and consequently incapable of causing disease in two distinct murine models of invasive pulmonary aspergillosis (IPA). Transcriptional profiling and annotation of genes that are affected by loss of SrbA function implicated that SrbA is involved in maintaining sterol biosynthesis and cell polarity. Further examination of the SrbA null mutant phenotype revealed that SrbA plays a critical role in ergosterol biosynthesis, resistance to the azole class of antifungal drugs, and in maintenance of cell polarity in *A. fumigatus*. Significantly, the SrbA null mutant was highly susceptible to fluconazole and voriconazole. These findings present a new function of SREBP proteins in filamentous fungi, and demonstrate for the first time that hypoxia adaptation is an important virulence attribute of pathogenic molds. (Poster # 473)

***Aspergillus fumigatus* gene expression in experimental murine lung infections**

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Aspergillus fumigatus is the most frequent cause of invasive aspergillosis in immune suppressed human patients. We have developed a murine model for analyzing the early stages of A. fumigatus colonization and progression to invasive disease. The model incorporates instillation of conidia into mouse lungs and subsequent harvesting of bronchoalveolar lavage fluid (BALF) samples for analysis. Validated mRNA amplification and analysis protocols have allowed transcriptome analysis of the fungal mRNAs present in the BALFs.
Expression profiling of *A. fumigatus* germlings at 12-14 hours after instillation into neutropenic mouse lungs reveals dramatically altered gene expression relative to growth in laboratory culture. Up-regulated genes are often found in secondary metabolism and other accessory gene clusters such as the gliotoxin, pseurotin, and siderophore biosynthesis clusters. We found also significant concordance between the observed host-adapted changes in the transcriptome and those resulting from *in vitro* iron limitation, nitrogen starvation, and loss of the LaeA methyltransferase.
To further elucidate the role of LaeA in *A. fumigatus* virulence, we analyzed temporal gene expression profiles of a wild type and an isogenic *laeA*-deleted strain, which misregulates gene expression at secondary metabolite gene clusters and is avirulent in a murine model. Growth and differentiation during initiating phases of murine infection were compared between parental and mutated isolates at 4, 8, and 14 hours post-infection in neutropenic mice. Transcriptome analysis of the *laeA* mutant revealed a major *in vivo* regulatory deficit of a few secondary metabolite biosynthetic gene clusters and more than thirty accessory gene clusters.
In our continuing studies employing this murine early infection model, we will undertake analysis of hypervirulent *A. fumigatus* mutants, *laeA* proteome analysis, and the murine host response to the fungal pathogen. (Poster # 450)

Ferroxidases in *Cryptococcus neoformans*: their roles inside macrophages and in antifungal susceptibility.

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Iron acquisition is a critical aspect of the virulence of many pathogenic microbes during growth within a mammalian host, and iron limitation is considered a primary defense mechanism by host cells. We study iron acquisition systems and iron-related regulatory mechanisms in the human fungal pathogen *Cryptococcus neoformans*. Our recent major discovery was the central role of the iron regulatory protein Cir1 in virulence, and we have also characterized a siderophore transporter Sit1 as well as the iron permeases Cft1 and Cft2. We recently identified the *Cryptococcus* ferrioxidases Cfo1 and Cfo2 that are coupled with Cft1 and Cft2, respectively, in the reductive iron uptake system. Here we present data that identifies specific roles for Cfo1 and Cfo2. Cfo1 is required for the reductive iron uptake system that is responsible for utilization of transferrin, a known primary iron source for *C. neoformans* within a host. However, Cfo1 did not play any role in heme or siderophore utilization. We constructed GFP fusion proteins and found that expression of Cfo1 is induced by iron limitation and is mainly localized at the plasma membrane. Furthermore, we found that Cfo1 is preferentially expressed within murine macrophages and that the catalytic subunit of cAMP-dependent protein kinase (Pka1) influences its localization *in vivo*. Strains lacking *CFO1* displayed increased sensitivity to antifungal drugs fluconazole and amphotericin B. Interestingly, wild type sensitivity was restored by addition of exogenous heme. These results suggested that increased sensitivity is due to the reduced levels of intracellular heme, which is required by proteins of the ergosterol synthesis pathway. Finally, the *cfo1* mutant was attenuated for virulence in a mouse model of infection thus revealing the significance of Cfo1 in pathogenesis. (Poster # 546)

Giant Cell virulence in Cryptococcus

Kirsten Nielsen, University of Minnesota

Cryptococcus neoformans is a common life-threatening opportunistic human pathogen that is ubiquitous in the environment. Humans are thought to be exposed to the organism by inhalation of spores. In healthy individuals the initial infection is either cleared or contained in granulomas. However, in immunocompromised individuals the infection can become severe and yeast cells can spread to the central nervous system resulting in meningitis. Early reports suggested the presence of enlarged cells in human and animal infections yet the identity of these cells and their role in virulence was unknown. Cryptococcal cells are typically 7-10 mm in diameter when grown *in vitro*. The majority of cells *in vivo* are 5-7 mm in diameter. However, cells 20-100 mm in diameter are observed in both human specimens and in the murine inhalational model of cryptococcosis. These enlarged cells, which we have termed “titan” cells, account for approximately 20% of the cells in the lungs by 72 hours post-infection. Titan cell production can be stimulated by coinfection with strains of opposite mating type and can be regulated by pheromone signaling. Characterization of titan cell interactions with host immune cells revealed reduced phagocytosis of the titan cells compared to normal cells, which may make them more resistant to host defenses. Titan cells are uninucleate and continue to bud and produce daughter cells *in vivo*. These results suggest that titan cells may represent an altered growth state which could be important for survival in the host environment.

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Candida albicans cell surface superoxide dismutases degrade host- derived reactive oxygen species to escape innate immune surveillance

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The clinical spectrum *Candida* spp. ranges from mucocutaneous infections to systemic, life-threatening diseases in immunocompromised patients. One of the immediate early responses of host phagocytes challenged by fungal pathogens is the production of reactive oxygen species (ROS), which are important in inflammatory reactions but also aim at destroying invading pathogens. Using quantitative real-time ROS assays based on chemiluminescence, we show here that both yeast and filamentous forms of the opportunistic human fungal pathogen *C. albicans* trigger ROS production in primary innate immune cells such as macrophages and dendritic cells. Through a reverse genetic approach, we demonstrate that co-culture of macrophages or myeloid dendritic cells with *C. albicans* cells lacking the superoxide dismutase Sod5 leads to massive extracellular ROS accumulation in vitro. Notably, ROS accumulation was further increased in co-culture with fungal cells devoid of both Sod4 and Sod5. Survival experiments show that *C. albicans* mutants lacking Sod5 and Sod4 exhibit a severe loss of viability in the presence of macrophages in vitro. The reduced viability is not evident with macrophages from *gp91phox*$^{-/-}$ mice defective in the oxidative burst activity, demonstrating a ROS-dependent killing activity of macrophages targeting fungal pathogens. These data show a physiological role for cell surface SODs in counteracting the oxidative burst reaction, and suggest a mechanism whereby *C. albicans*, and perhaps many other microbial pathogens, can evade the host immune surveillance in vivo. (Poster # 522)

A filament-specific transcriptional regulator determines _Candida albicans_ morphology and virulence

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Candida albicans, the major human fungal pathogen, undergoes a reversible morphological transition from single ovoid yeast cells to pseudohyphal and hyphal filaments. In order to address the mechanisms that determine *C. albicans* morphology and virulence, we have generated a strain that can be genetically manipulated to grow completely in the hyphal morphology under non-filament-inducing conditions *in vitro*. This was achieved by inducing high-level constitutive expression of *UME6*, a recently identified filament-specific transcriptional regulator of *C. albicans* hyphal extension. We show that high-level *UME6* expression significantly increases hyphal formation and promotes virulence during the course of infection in a mouse model of systemic candidiasis. We also demonstrate that lower levels of *UME6* expression specify growth largely in the pseudohyphal form and that increasing *UME6* levels is sufficient to cause cells to gradually shift from pseudohyphal to hyphal morphology; an accompanying increase in both the number of filament-specific genes expressed, as well as their level of induction, is observed. These findings suggest that a common transcriptional regulatory mechanism functions to specify both pseudohyphal and hyphal morphologies in a dosage-dependent manner.

Understanding sex in a hostile environment - cryptic mating in _Candida albicans_

Richard J. Bennett, Brown University, RI.

The opportunistic human pathogen *Candida albicans* was, until recently, thought to be an obligate asexual fungus. A cryptic mating cycle has been uncovered, however, in which efficient mating requires that cells first undergo a phenotypic switch from the 'white' state to the mating competent 'opaque' state. This novel form of mating regulation is thought to limit *C. albicans* mating to specific host niches. Evidence from the laboratory demonstrates that the white-opaque switch is highly sensitive to diverse environmental stimuli, including oxidative and genotoxic stress, as well as genetic manipulation of strains. We show that these apparently diverse factors influence the rate of phenotypic switching via an effect on the rate of cell growth. A model is discussed whereby changes in growth rates alter the frequency of switching by modulating Wor1 protein levels – the central protagonist for formation of the opaque state in *C. albicans*. Increased phenotypic switching in response to stressful environments may be beneficial to the organism as it can directly promote escape from host defenses, as well as directing entry into the program of sexual reproduction.

Population genetics and ecology: Lynne Boddy and Kwangwon Lee

Dispersal as a key to fungal population biology: creating wind, and travelling with humans.
Anne Pringle, Hugh Cross, Franck Richard, Marcus Roper, Agnese Seminara, Benjamin Wolfe. Department of Organismic and Evolutionary Biology, Harvard University

Dispersal is a crucial parameter in models of phylogeography and population biology. Although fungi do possess adaptations for active dispersal, dispersal is more often perceived as a passive dependence on water or wind. By integrating perspectives from physics and biology I will explore mechanisms of especially ascomycete dispersal and suggest that most species of fungi have evolved to manipulate their physical environments. For example, ascomycetes that "puff" are literally creating their own wind. I will end by discussing the role of humans in fungal dispersal: as fungal species are moved across and among continents, traditional barriers to dispersal are crossed and novel opportunities for interactions among populations and species are created.

Whole genome sequencing of experimental yeast populations identifies determinants of divergent adaptation and reproductive isolation
James B. Anderson, Caroline Sirjusingh, Jeremy Dettman, and Linda M. Kohn

Understanding how speciation occurs is a central goal of evolutionary biology. The link between divergent selection in isolated populations and the onset of reproductive isolation was recently demonstrated in experimental populations of the yeast Saccharomyces cerevisiae. In this system, reproductive isolation had two independent origins, ecological isolation evident as phenotypic mismatch of hybrids to the environments in which selection occurred and Muller-Dobzhansky (MD) interactions evident as inherent genetic conflict among adaptive mechanisms, independent of environment. Still lacking in any system was a genome-wide identification of the determinants of adaptation and reproductive isolation. Here we deployed whole-genome sequencing to identify candidate mutations in two haploid representatives from yeast populations evolved for 500 generations in a high-salt environment and two from a low glucose environment. From these candidates, we discovered the mutations responsible for the adaptive increases in mitotic fitness and for the reproductive isolation in hybrids.

Evolution of the mating type chromosome in *Neurospora tetrasperma*
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Neurospora tetrasperma is unique among species of *Neurospora* in that, after meiosis, two haploid nuclei of opposite mating type are packaged into a single ascospore. This pseudohomothallic condition results in self-fertility. Blocked recombination proximal to the mating type locus during meiosis I is necessary for the correct packaging of alternative mating type alleles into a single heterokaryotic ascospore. Previous genetic studies have shown that recombination is suppressed along the majority of the mating type chromosome (75% / 7Mbp) possibly due to rearrangements within the *N. tetrasperma mat A* chromosome. The Joint Genome Institute has recently sequenced both mating types of *N. tetrasperma* (*mat a* [FGSC #2509] and *mat A* [FGSC #2508]) at 0.5X and 8X respective coverage. In addition, two runs of 454 pyrosequencing (one standard and one paired-ended) were completed for *N. tetrasperma mat A*, increasing coverage to 23X and dramatically improving the assembly. Here we show that a series of intrachromosomal rearrangements have occurred on the *N. tetrasperma mat A* chromosome with respect to *N. crassa mat A*, while the autosomes remain essentially collinear. We suggest that these rearrangements may be contributing to the *mat* chromosome recombination block and we plan to investigate the characteristics of the identified breakpoints.

From population genetics to population genomics in wood decay fungi
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Traditionally, population genetic studies have been based on allelic variation at a limited number of loci. Most studies have utilised neutral or near neutral markers for inferring geographic structure or demographic differences that sometimes have been compared to life history traits. With a lower cost for and an increasing access of DNA sequence data whole genome sequences can be used for inferring population structure. In addition to giving a much stronger statistical support for the results, the approach opens up for scans for information of linkage disequilibrium, signs of selection, genetic mapping of functional traits, subspecies detection etc. This presentation will give examples of traditional population genetics work in decay fungi by using whole genome sequence data from several individuals within the *Heterobasidion annosum* species complex to show how population genomic work might develop to ask and answer questions on species phylogeny and on evolution of neutral and adaptive traits. (Poster # 434.)

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Evolutionary history of the mitochondrial genome in *Mycosphaerella* populations infecting bread wheat, durum wheat, and wild grasses.

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The recent release of the complete mitochondrial genome of *Mycosphaerella graminicola* (EU090238) provided a tool to infer the evolutionary history of *Mycosphaerella* populations infecting bread wheat (*Triticum aestivum*), durum wheat (*Triticum durum*) and wild grasses (*Lolium multiflorum, Dactylis glomerata* and *Agropyron repens*). Analyses of DNA sequence data from nuclear loci indicated that *M. graminicola* emerged as a new pathogen adapted to wheat from an ancestral population infecting wild grasses in the Middle East during the process of wheat domestication around 10,500 years ago. The evolutionary history of the mitochondrial genome inferred from four mitochondrial loci (total of 1338 bp) dated the divergence of *M. graminicola* from the ancestral *Mycosphaerella* populations between 10,200 and 9,000 years ago, confirming the coalescent analysis conducted using nuclear markers. The previously proposed non-random association between mitochondrial genome haplotypes of *M. graminicola* and host-specific populations infecting bread wheat or durum wheat was considered after identifying a 1935 bp putative gene that is exclusive to a mtDNA haplotype that is found at a higher frequency on durum wheat. The putative protein from this gene was predicted to have ligase activity based on its amino acid sequence. It was noted earlier that ligases could act as virulence factors that are able to manipulate host metabolism to overcome plant innate immunity.

"Out of Africa" origin of human pathogenic fungus *Cryptococcus neoformans* var. *grubii*.

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Cryptoccocus neoformans var. *grubii* (serotype A) is the most common cause of fungal meningoencephalitis, one of the most prevalent and deadliest fungal infections in humans, which has a particularly devastating effect on AIDS patients in sub- Saharan Africa. Although this pathogen is ubiquitous around the world, yeast population in southern sub-Saharan Africa is genetically different from the global population. Here we present evidence that African population of the pathogen has a unique ecological niche in endemic African trees. We demonstrated that this niche harbors the ancestral yeast population, which represent s the evolutionary hotbed and center of speciation of *C. neoformans* var. *grubii*. We also demonstrate that global population of this fungus originated from a single expansion of two strains from the ancestral population in Africa, which became associated with the pigeon guano and were spread around the world by migration of humans and pigeons. (Poster # 423)

Fungal diversity in deep-sea sediments - a whole new world?

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Deep-sea environment remains one of the most unknown research areas, holding great potential and interest for science. There have been many reports on the discovery of new organisms, life cycles and bio-resources from deep-sea environments. However, whereas the prokaryotic components of known deep-sea related organisms have been extensively described, very few studies have been focused on fungal communities associated with deep-sea environments. In this study, we examined the fungal diversity of 11 different deep-sea sediment samples (depth range between 200-10,000m) by amplifying the internal transcribed spacer (ITS) regions of rRNA genes with fungal- specific PCR primers. A total of 11 ITS libraries were constructed and 1056 clones were selected randomly. As a result of this study, some common fungal species in surface environments, such as *Penicillium*, *Aspergillus*, *Trichosporon* and *Candida* were identified. However, the majority of amplified ITS sequences were not associated with any known fungal sequences in the public database. Phylogenetic analyses suggested that some of these sequences could be included in the order of Chytridiomycota. Another interesting result was that one sequence was detected in all fungi-positive samples, despite the locality and depth difference. The most closely related fungal species of the sequence was *Metschnikowia colocasiae* (70% similarity). The results indicated that deep-sea sediments harbor diverse fungi, including new taxonomic groups that may be relevant to the early evolution of fungi and the discovery of new microbial metabolites. (Poster # 407)

Fungal insect competition and the secondary metabolism

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Filamentous fungi and saprophage insects are suspected to be competitors on decaying organic matter. Both organisms have equal requirements considering habitat and nutrition. Insect larvae negatively influence mould development (1), but filamentous fungi can be an important cause of mortality of insect larvae (2). These competitions in insect- mould interactions have largely been ignored. First investigations suggest a role of genes for fungal secondary metabolism (3). We ought to be investigate the function of moulds secondary metabolites as a chemical defence in insects-moulds-interactions as well as the influence of these competitors at trophic interaction between insects . Microarrays of secondary metabolism genes of *A. nidulans* are being used to identify fungal target genes up- or downregulated when interacting on festered matter with the antagonistic Drosophila larvae. Preliminary tests employing real time RT-PCR with RNA from *A. nidulans* confronted with *D. melanogaster* larvae indicates upregulation of the global regulator laeA, as well as aflR and sterigmatocystin biosynthesis genes. The consequence on evolutionary fitness of fungi and insects will be discussed.

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Secondary metabolism: Barbara Howlett and Jurgen Wendland

Oxidative stress response and riboflavin production in *Ashbya gossypii*

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Ashbya gossypii (Eremothecium gossypii) is a filamentous fungus belonging to the family of *Saccharomycetaceae*. It is a known overproducer of riboflavin (vitamin B2). *Eremothecium cymbalariae*, a close relative of *A. gossypii* shows some morphological distinctions and does not overproduce riboflavin. It is known that addition of cAMP to the medium inhibits riboflavin production in *A. gossypii*. An *A. gossypii* strain deleted for *SOK2*, which encodes a transcription factor that plays a regulatory role in the cyclic AMP (cAMP)-dependent protein kinase (PKA) signal transduction pathway, results in strains that shows no riboflavin overproduction and appears white. Strains producing less riboflavin show an increased sensitivity against H_2O_2. In *S. cerevisiae* Yap1 controls the expression of genes in response to oxidative stress. In our effort to understand the biological role of riboflavin overproduction we, therefore, analyzed the oxidative stress response of *A. gossypii*. We generated an *Agyap1* mutant strain, which was found to produce less riboflavin than the parental strain. The sensitivity of *Agyap1* against H_2O_2 and menadione was increased. Using *lacZ* as reporter we found that Yap1 regulates the expression of *RIB4*, which encodes a gene of the riboflavin biosynthesis pathway. Comparison of AgYap1 with other fungal Yap1 proteins, e.g. *S. cerevisiae Yap1*, showed that AgYap1 lacks the characteristic cysteine rich domains. Surprisingly, these domains were found in the *E. cymbalariae* Yap1. A Hybrid Ag/Sc-Yap1 protein with the C-terminal cysteine rich domains of ScYap1 could complement the *Scyap1* H_2O_2 sensitivity, while AgYap1 could not. In conclusion, *A. gossypii* harbours a unique *YAP1* gene, which links the oxidative stress response with riboflavin production.

Delving into the mechanism of LaeA regulated secondary metabolism

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LaeA, a global regulator of secondary metabolism within the aspergilli and related fungi, has been shown to be important for pathogenicity in a model of invasive aspergillosis. Null mutants of LaeA are nearly avirulent in the murine model and also show increased phagocytosis by alveolar macrophages *in vitro*. LaeA has recently been shown to be part of a protein complex, termed the velvet complex, which coordinates sexual development and secondary metabolism in the model organism *A. nidulans*. While LaeA control of secondary metabolism appears to be conserved within the genus *Aspergillus*, its function remains an enigma. We are interested in determining what makes LaeA contribute to pathogenicity in *A. fumigatus*. Towards this end, we have constructed a normalized yeast-two hybrid cDNA library to screen for protein interactors of LaeA. Additionally, we have utilized an AMA1 genomic library of *A. fumigatus* (AF293) to look for multi-copy suppressors of *?laeA*. By employing two forward genetic screens in the pathogenic mold *A. fumigatus*, we have putatively identified several loci that may be involved in LaeA regulated pathogenicity factors. (Poster #483)

Dothistromin biosynthesis in the pine-infecting fungus, *Dothistroma* spp.

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Dothistromin is a non host-specific toxin produced by several fungi including the pine needle pathogen *Dothistroma septosporum*. It is similar in structure to versicolorin B, an aflatoxin precursor, and is produced in culture and *in planta*. The red bands typically seen in *Dothistroma*-infected pine needles are principally due to accumulation of this toxin. Injection of purified dothistromin into needles led to development of typical disease symptoms, suggesting a major role in disease. However, studies with toxin-deficient mutants of *D. septosporum* showed that dothistromin is not required for pathogenicity. Mutant strains were able to colonise needles, trigger lesion formation and sporulate the same as dothistromin-producing strains. Dothistromin is unusual among fungal secondary metabolites in being produced at a very early stage of growth, rather than in late exponential or stationary phase. The early onset of dothistromin production, along with its broad-spectrum toxicity, led to our current hypothesis that dothistromin has a role in protection against other microorganisms in the needle environment. *In vitro* studies with needle-dwelling organisms support this. We further propose that dothistromin mainly functions to inhibit growth of other microorganisms within the needle, such as endophytes and latent pathogens, and will present our reasoning for this speculation. (Poster # 448)

***Metarhizium anisopliae NPS1* synthesizes the novel cyclic heptapeptide spore factors called serinocyclins.**

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Metarhizium anisopliae is at the forefront of efforts to develop entomopathogenic fungi as insect biocontrol agents and has been used worldwide for invertebrate pest control as an alternative to chemical pesticides. Numerous secondary metabolites have been described from the fungus, including the well-known cyclic depsipeptide destruxins, but the roles of these compounds as predicted virulence factors in disease development are poorly understood. We targeted for disruption by *Agrobacterium tumefaciens*-mediated transformation a putative nonribosomal peptide synthetase (NPS) gene, *MaNPS1*, which is expressed concurrently with destruxin biosynthesis. Analyses of three *manps1* mutants showed that destruxin production was unaffected. Instead, a novel family of cyclic heptapeptides, the serinocyclins, was detected in extracts of conidia of control strains but not in conidia of *manps1* mutants or in other developmental stages, suggesting that *MaNPS1* encodes a serinocyclin synthetase. *Manps1* mutants exhibited in vitro development and responses to external stresses comparable to control strains. No detectable differences in pathogenicity of the mutants were observed in bioassays against beet armyworm and Colorado potato beetle. However, purified serinocyclin A disrupted the normal swimming behavior of mosquito larvae. This is the first report of targeted disruption of a secondary metabolite gene in *M. anisopliae*, which revealed a novel cyclic peptide spore factor. The fusarin-like mutagens NG-391 and NG-393 also were described from the fungus for the first time by analysis of a serinocyclin-deficient mutant that secreted ca. 10-fold more of the yellow pigments into culture medium than did control strains.

Analysis of secondary metabolite gene clusters in the genome of *Epichloë festucae*

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Epichloë festucae is a model endophytic fungus for which the genome has recently been sequenced. Analyses based on protein domain structures revealed 29 clusters of biosynthetic gene models coding for known or predicted secondary metabolites in the fungal genomic sequence data. Most of the clusters contained a polyketide synthase gene, nonribosomal peptide synthetase gene, or both, although these were not featured in the loline alkaloid cluster (*LOL*). We investigated linkages between the secondary metabolite biosynthetic genes and clusters, their distribution throughout the fungal genome, and their possible telomeric associations. We conducted long-range mapping of putative secondary metabolite gene clusters and telomeres on *Not*I restriction fragments from fungal genomic DNA. Preliminary results suggested that several of the clusters are linked to form clusters of clusters. Even more intriguing, a third of the identified clusters appear to be located in subtelomeric regions. Both the ergot and loline alkaloid biosynthesis gene clusters appear to be subtelomeric. Unique for fungal genomes, we found that more than 50% of the telomeres are associated with secondary metabolite gene clusters, which may be important in regulation of pathway gene expression, or to maintain their polymorphism in populations. (Poster # 353)

SMURF: genomic mapping of fungal secondary metabolite pathways

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Filamentous fungi produce an impressive array of secondary metabolites (SMs) including mycotoxins, pigments, antibiotics and pharmaceuticals. The genes responsible for SM biosynthesis, export, and transcriptional regulation are often found in SM gene clusters. We developed the web-based software SMURF (Secondary Metabolite Unknown Regions Finder) available at www.jcvi.org/smurf/ to search systematically for such clusters. SMURF's predictions are based on the domain content of genes located close to backbone genes that often encode the first steps in SM pathways. In tests, SMURF accurately recovered all known SM clusters and detected 8 additional potential clusters in *Aspergillus fumigatus*.

We applied SMURF to catalog putative SM clusters in 25 publicly available fungal genomes. Among these taxa, the Aspergilli appear to have the coding capacity for the largest numbers of these metabolites. The genome of *A. niger* alone encodes 61 backbone enzymes and 58 clusters. Further comparison reveals that SM pathways are very unevenly distributed among fungal taxa suggesting an evolutionary pattern of rapid pathway gain and loss. It also confirmed the correlation between unicellularity and the absence of SMs, and the prominent role of gene duplication in the creation of new pathways. (Poster # 91)

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The transcription factor FgStuA influences spore development, pathogenicity and secondary metabolite production in *Fusarium graminearum*.

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Members of the APSES family of fungal proteins have been identified as key regulators of fungal development, controlling processes such as mating, sporulation and dimorphic growth. We deleted the *FgStuA* gene in *Fusarium graminearum* and show that the mutant is greatly impaired in spore development, pathogenicity and secondary metabolism. *FgStuA* is closely related to *FoStuA* in *F. oxysporum*, but unlike *FoStuA* mutants the *FgStuA* mutants were greatly reduced in pathogenicity both on wheat and apple slices. The lack of ability to cause disease on wheat heads may be due to lack of trichothecene accumulation *in planta*. The *FgStuA* mutant also had a white/yellow mycelial phenotype compared to the red pigmented (aurofusarin) wild-type, had reduced aerial mycelium, susceptibility to oxidative stress, and had a less hydrophobic surface. Microarray analysis showed that most phenotypes could be inferred from gene expression data, such as down-regulation of the trichothecene gene cluster in the mutant. In an attempt to separate primary and secondary effects of *FgStuA* deletion, we carefully examined gene expression data together with promoter analysis and comparative genomics. The genes flanking *FgStuA* are conserved and syntenous in other fungal genomes and contain a gene encoding a putative clock controlled protein. FgStuAp and other APSES proteins share significant homology with DNA-binding domains of transcription factors controlling the critical G1/S phase cell cycle transition in both *S. cerevisiae* and *S. pombe*. Genes within MIPS Functional Category (FunCat) 10 “Cell cycle and DNA processing” are enriched among those more highly expressed in the *FgStuA* mutant than wild-type. *Aspergillus* StuAp response elements (A/TCGCGT/ANA/C) also were found highly enriched in promoter sequences for FunCat 10 genes, compared to the genome as a whole. Our results suggests that FgStuAp may act primarily as a repressor involved in cell cycle regulation, and may act only secondarily on sporulation, pathogenicity, and secondary metabolism. (Poster # 512)

Discovery of the emericellamide gene cluster by genomic mining in *Aspergillus nidulans*

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The recently sequenced genomes of several *Aspergillus* species, including *A. nidulans*, have revealed that these organisms have the potential to produce a surprising large range of natural products. Exploiting this discovery will, in turn, depend on advancements in tools for manipulating *Aspergillus* genomes and on understanding *Aspergillus* secondary metabolite regulation. We have employed recently developed gene targeting procedures, in combination with natural products chemistry, to discover novel nonribosomal peptide synthetase (NRPS)/polyketide synthase (PKS) gene cluster that synthesizes emericellamides. We further establish the boundaries of the eas (emericellamide synthesis) gene cluster and propose a biosynthetic pathway through a series of targeted deletions. The identification of this cluster opens the door to engineering novel analogs of these complex metabolites. (Poster # 354)

Applied genomics and industrial mycology: Ken Bruno and Peter Punt

Exploiting new sequencing technologies to study fungal genome structure and variation

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We have applied new sequencing technologies that generate large amounts of inexpensive data to genome assembly and polymorphism discovery in fungi. In doing so, we have developed new methods to maximize the utility of these new data types.
ALLPATHS is a graph theory-based Illumina read assembly algorithm that delivers highly accurate and contiguous assemblies for genomes of up to 40Mb in size, e.g. Schizosaccharomyces pombe, Candida albicans and Neurospora crassa. For example, our ALLPATHS assembly of S. pombe yields contig and scaffold N50 sizes of 230kb and 1.3Mb respectively (base accuracy >Q50) and covers 98% of the reference. Further, using 454 data exclusively or in combination with Sanger data we generate quality assemblies and can capture large regions of the genome absent from Sanger libraries, e.g. Neurospora crassa. For polymorphism discovery, we have developed VAAL, an algorithm that uses Illumina data to identify differences between microbial genomes at high sensitivity and specificity. VAAL typically detects ~98% of differences (SNPs to large indels) between pairs of related strains of bacteria while calling no false positives and is sensitive enough to pinpoint a single drug resistance mutation between bacterial genomes e.g. Vibrio. We have also used VAAL to identify polymorphisms in strains of Schizosaccharomyces and are applying this methodology to larger fungal genomes. (Poster #3).

Systems biology-based analysis of the response of *Aspergillus niger* to ambient pH

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The filamentous fungus *Aspergillus niger* is an exceptionally efficient producer of organic acids which is one of the reasons for its relevance to industrial processes and commercial importance. While it is known that the mechanisms regulating this production are tied to the levels of ambient pH, the reasons and mechanisms for this are poorly understood. To cast light on these complex systems, we integrate results from two genome-based strategies: A novel method of genome-scale modeling of the response, and transcriptome analysis across three levels of pH.
Using genome scale modeling with an optimization for extracellular proton-production, it was possible to reproduce the preferred pH-levels for citrate and oxalate. Transcriptome analysis and clustering expanded upon these results and allowed the identification with distinct transcription patterns across the different pH-levels examined. New and previously described pH-dependent *cis*-acting promoter elements were identified. . Knock-out mutants for a number of pH-dependent transcription factors have been constructed and preliminary characterization results will be presented. Integration of regulatory profiles with functional genomics led to the identification of candidate genes for all steps of the *pal/pacC* pH signalling pathway
The combination of genome-scale modeling with comparative genomics and transcriptome analysis has provided systems-wide insights into the evolution of highly efficient acidification. It has also made clear that filamentous fungi have evolved to employ several offensive strategies for out-competing rival organisms.

Comparative functional genomics: Reconstructing the evolution of central carbon metabolism in 15 fungal species

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Molecular networks are the information processing devices of cells and organisms. Networks are remarkably flexible and can re-configure in an adaptive response to perturbation. We seek to understand the mechanisms by which molecular networks accommodate changes by analyzing genomics data through the unifying abstraction of the functional module. Specifically, we focus on evolutionary changes that occur in the organization and regulation of central carbon metabolism in *Ascomycota* fungi. Although central carbon metabolism follows the same general theme in all yeasts, important biochemical, genetic and regulatory variations exist. For example, physiologically yeasts can be classified into respiro-fermentative and respiratory during aerobic growth on glucose. We constructed a genomics platform to study a set of 15 fully sequenced yeast species spanning over 300 million years of evolution. We grow each organism under a variety of conditions (e.g. rich glucose, alternative carbon sources) and along transitions from one condition to another. We measure a variety of phenotypes: growth characteristics, global expression, metabolomics and proteomics profiles. We use our computational algorithms for orthology mapping (SYNERGY) and regulatory network reconstruction (CISPROF), along with novel algorithms, to reconstruct the modules in each extant species, and those that existed in ancestral ones. We then mine those reconstructions for general patterns of how module content and regulation evolves and how changes occur at different molecular levels (transcription, protein, metabolic). Analysis of global expression and metabolite profiles from experiments in which the cells are grown until the glucose is depleted and alternate carbon sources are metabolized revealed both universal (e.g. repression of ribosomal genes) and species-specific (e.g. differential regulation of oxidative phosphorylation) trends. We have reconstructed many of the ancestral modules of this group giving us unique insight into the evolutionary process. (Poster # 14)

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Activation of silent gene clusters in *Aspergillus nidulans*

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Microorganisms as bacteria and fungi produce important low-molecular weight molecules that show different biological activities. Genome mining of available fungal genomes indicated that their potential to produce these compounds designated secondary metabolites (SMs) is greatly underestimated. Fungi encode the genetic information for the biosynthesis of many more compounds which still await discovery. The vast amount of DNA sequence in the public database represents only the beginning of this new genomics era. Most of the fungal secondary metabolism gene clusters are silent under laboratory conditions. Despite this limitation, to get access to the vast number of unknown compounds encoded by silent gene clusters, mixing genomic data, genetic engineering and analytical techniques provides a new avenue to discover novel and potentially bioactive natural products. Bioinformatic analysis of the published *A. nidulans* genome sequence led to the identification of 48 putative SM gene clusters. By overexpressing a pathways-specific transcription factor, we were able to induce a silent gene cluster containing the gene for a mixed PKS/NRPS system. This approach is rendered feasible by the fact that all of the genes encoding the large number of enzymes required for the synthesis of a typical secondary metabolite are clustered and that in some cases, a single regulator controls the expression of all members of a gene cluster to a certain extent. By this way, we were able to isolate novel compounds. In addition, we will discuss further attempts to activate silent gene clusters and to identify physiological conditions under which gene clusters are active under natural conditions.

An archeological exploration of fungal production strains: Analysis of *Aspergillus niger* AMG Producing Strains Using Comparative Genome Hybridization (CGH) and Quantitative Real-Time PCR (qPCR).

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Over the past few decades, *Aspergillus niger* has been improved for glucoamylase production by classical mutagenesis. In order to begin characterizing these strains to determine what changes have lead to the increased productivity, microarray-based comparative genome hybridization (CGH) analysis was used to obtain information regarding genomic changes that occurred during successive rounds of mutagenesis . This analysis revealed both deletions and amplifications of discrete chromosomal segments ranging in size from less than one kb to as much as 400 kb. Most importantly, we observed successive amplifications of a 216 kb region that included the *glaA* (glucoamylase) gene in the AMG strain lineage. In addition, CHEF gel analyses suggest that the amplified DNA segment has also undergone several non-reciprocal translocations in the AMG strain lineage. Quantitative PCR analyses confirmed the CGH results, leading us to conclude that at least some of the incremental improvements in AMG titers obtained from mutants derived by successive rounds of mutagenesis and screening may be associated with increases in the number of chromosomal *glaA* gene copies.

A systems biology approach towards improvement of itaconic acid production in Aspergillus sp.

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Filamentous fungi are widely used for enzyme and metabolite production for a wide variety of uses - for example food, feed, textile, paper and pulp, fuels and chemicals, detergents - due to the development of extremely productive strains and production processes. With the advent of molecular genetic tools, in the last two decades very successful strain improvement programs for protein and secondary metabolites have been developed. However, for primary metabolites, such as organic acids, which are still by volume the largest fungal bioproducts, real breakthroughs have not been made in recent years. In our research we have addressed the production of one of the commercially interesting building-block organic acids, itaconic acid. To unambiguously identify the itaconic acid biosynthetic pathway several parallel approaches were taken using Aspergillus terreus as parental host strain. Using a combination of controlled fermentation design, reversed genetics and transcriptomics approaches the pathway specific cis-aconitate decarboxylase (CAD) encoding gene was identified. This gene encodes the enzyme required to make itaconic acid from general TCA cycle intermediates. In addition to this gene several other target genes for improvement of itaconic acid production were identified.
A highly relevant issue for the bioproduction of bulk-products like organic acids is also the cost of production. As production costs rely for 30-60% on the cost of feedstock, in frame with our itaconic acid project we also evaluated performance and feedstock versatility of several potential microbial production hosts on lignocellulosic substrates. From this analysis Aspergillus niger was selected as preferred host.

Engineering intracellular metabolism by altering gene expression of *Aspergillus oryzae*

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Aspergillus oryzae is one of the most important organisms in Japanese fermented food industry. Though it hardly produces secondary metabolites, related organisms are producers of diverse metabolites. A major objective of our project is to develop a system using *A. oryzae* to generate diverse metabolites. Combinatorial biochemistry approach by introducing exo-genous genes and fermentation techniques are combined. To reveal regulation of metabolic pathways in *A. oryzae* under various conditions, gene expression profiles under condition of submerged culture, solid media and so on were analyzed by DNA micro array. A series of deletion mutants of transcription factor have been obtained by systematic gene targeting. More than 200 mutants were tested for their ability to produce some metabolites. Several mutants showed lack of production or different dependency on environmental nutrient conditions. The factors affecting expression level of metabolic genes are being studied. To develop the system, novel vectors and host strains of *A. oryzae* have been constructed. We have replaced promoter regions of some metabolic genes and successfully altered the level of some metabolite productions. To introduce genes, the correct detection of splicing position is important. We have analyzed motifs of splicing in *A. oryzae* by comparing EST and genome sequences. (Poster # 189)

Development of Chrysosporium lucknowense C1 as a commercial protein production platform: Exploration and exploitation of its genome.

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Several filamentous fungi can secrete large amounts of protein into the growth medium and are therefore commonly used as hosts for the production of industrial enzymes. We have developed the ascomycetous fungus *Chrysosporium lucknowense* C1 for protein production on a commercial scale as an alternative to well known fungi like *Aspergillus niger* and *Trichoderma reesei*. Strain and process improvement strategies of the original C1 isolate resulted in strains that are able to secrete large amounts of a complex mixture of (hemi-)cellulases. Additionally, these strains show a strong reduction in culture viscosity as result of a morphology change. The low-viscosity property allows for growing C1 to high density in fermenters, yielding very high protein production levels. Recently, the sequencing and automated annotation of the C1-genome was finished. Genome mining revealed an impressive enzymatic potential. Especially, the repertoire of genes encoding plant biomass hydrolyzing enzymes appeared overwhelming. Currently, this knowledge is being exploited: C1(hemi-) cellulases obtained by (selective) over-expression and by purification are tested for saccharification of (lingo-) cellulosic substrates. These studies provided leads towards the development of enzyme mixtures that will efficiently and cost-effectively convert residual biomass to fermentable sugars. An overview will be given covering the exploration and the exploitation of the C1-genomic potential in order to develop C1 as a commercial protein production microorganism. (Poster # 36)

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Architecture and tip growth:

Phosphorylation of Sec2 by Cdc28-Hgc1 is required for transport of secretory vesicles to the Spitzenkörper during the hyphal growth of *Candida albicans*

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Candida albicans hyphae grow in a strongly polarised fashion from the tip driven by a Spitzenkörper, a subapical body rich in secretory vesicles. It is thought that the Spitzenkörper acts as a supply centre ensuring that more vesicles fuse the plasmamembrane at the hyphal apex than fuse subapical regions. At sites polarised growth in *S. cerevisiae* a multiprotein complex called the exocyst mediates the docking secretory vesicles with the cell surface, while a second complex called the polarisome nucleates the formation of actin cables along which secretory vesicles are transported by a class V myosin, Myo2, partnered by its regulatory light chain Mlc1. Cytological studies using protein fusions to GFP show that Mlc1, Sec4 and Sec2 localise to a distinct intracellular apical spot resembling a Spitzenkörper, while exocyst and polarisome components localise to surface crescent. Thus during hyphal growth the Spitzenkörper is clearly a separate structure from the polarisome and exocyst. Transport of post-Golgi secretory vesicles to sites of polarised growth and their fusion with the cell surface requires the action of the Rab-family GTPase Sec4. Activation of Sec4 to its active, GTP-bound form is mediated by its guanine exchange factor Sec2. We reasoned that the accumulation of secretory vesicles in the Spitzenkörper during the switch to hyphal growth may be promoted by Sec2. In order to test this hypothesis we have dissected the regulation of the Sec2. We identified two sites where Sec2 is phosphorylated shortly after hyphal induction in a manner which is independent of the hyphal specific transcription program. Both of the phosphorylation events are required for normal hyphal development. Further we show that the cyclin-dependent kinase Cdc28 kinase, partnered by the hyphal specific cyclin, Hgc1, is responsible for phosphorylating one of these residues. Mlc1 physically interacts with Sec2 and the region on Sec2 where Mlc1 binds is different in the hyphal and yeast forms. Thus the accumulation of secretory vesicles in the Spitzenkörper requires Sec2 phosphorylation by Cdc28 which may act by altering the interaction of Sec2 with the motor complex that transports secretory vesicles to the hyphal tip.

The exocyst in *Neurospora crassa*: a tale of vesicles, fusions and apical growth.

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The last stage of the secretory pathway from the *trans*-Golgi to the plasma membrane (PM) is exocytosis, a process requiring high fidelity protein-protein interactions to fuse secretory vesicles to the PM. The exocyst is a conserved octameric complex consisting of SEC-3, -5, -6, -8, -10, -15, EXO-70 and -84. It marks the site of vesicle fusion to the PM, and is regulated by several GTPases (SEC-4, RHO-1, RHO-3, CDC-42). *Neurospora crassa* has single genes for all eight exocyst components. We constructed translational fusions of all exocyst genes with GFP, 3xHA and/or FLAG tags at their endogenous loci by a split marker "knock-in" procedure. The localization and dynamics of the GFP fusions was examined in living hyphae by laser scanning confocal microscopy. For all tagged exocyst components, GFP fluorescence accumulated primarily adjacent to the plasma membrane at the hyphal dome, which correlates with the place of intensive exocytosis in polarized growth. A low GFP fluorescence was observed around the pore of newly formed septa, while a strong fluorescence was noticeable at sites of hyphal contact in anastomosed hyphae and in older septa. By crosses and heterokaryon formation, we generated strains that carry exocyst genes with different tags and verified the expression of all fusion proteins by western blot. We are co-immunoprecipitating exocyst components to uncover exocyst assembly pathways and important interaction domains. Our cytological data suggest that the exocyst is involved in vesicle fusion during apical growth and during pore development both in septa and in fusing hyphae. (Poster # 290)

The *Aspergillus nidulans* cell end marker TeaA controls the processive microtubule polymerase XMAP215 and inhibits microtubule growth at the cortex

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During cellular morphogenesis among the different cell types, microtubules deliver positional information to the proper site of cortical polarity. Once microtubules and their associated proteins determine the polarity site, a positive feedback loop initiates to reinforce and maintain the polarity site through rearrangement of actin cytoskeleton. To perform the function, microtubules have to search a specific site at the cortex and stop elongation once they reach the cortex. Here, we show in *Aspergillus nidulans* that the contact of microtubule plus ends with the cortex is mediated through protein-protein interaction between a processive microtubule polymerase (XMAP215, *A. nidulans* homologue AlpA), and a cortical cell end marker protein, TeaA. Although both proteins localized to the microtubule plus end during microtubule growth, AlpA-TeaA interaction was observed only after contact with the cortex. In the absence of TeaA, microtubules continued to grow after reaching the cortex. In the absence of AlpA, the microtubule array was largely affected and microtubules grew very slowly. To determine if AlpA polymerase activity is directly controlled by the interaction with TeaA, we developed an *in vitro* assay system. Our results suggest that microtubule polymerization depends on the presence of AlpA and growth is inhibited by TeaA.

Spitzenkörper distribution and diversity among the fungi.

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Hyphal growth and morphogenesis are complex processes that have allowed the fungi to successfully utilize a wide range of ecological habitats and develop multiple lifestyles. Bioimaging studies, and more recently molecular studies, of hyphal tip biology have placed great emphasis on the Spitzenkörper. The Spitzenkörper appears to have evolved only in filamentous fungi, where it is present in all members of the Basidiomycota and Ascomycota studied thus far. Spitzenkörper have been verified only in a few members of the 'lower fungi.' Though progress is being made towards better understanding the cellular and molecular biology of the Spitzenkörper, important details remain unclear regarding its structural diversity and distribution among the filamentous fungi. In this presentation, light and electron microscopy are used to address Spitzenkörper structure and distribution among fungal taxa.

BEM-1 is required for directed but not for general polar growth in *Neurospora crassa*

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The molecular mechanisms mediating cell cell fusion are only poorly understood. We are using *Neurospora crassa* as a model system to study cell fusion and related signaling mechanisms. Germinating *N. crassa* spores show chemotropic growth towards each other and fuse. As a result individual germlings become one functional unit, which subsequently develops into the mycelial colony. Earlier studies have shown that the MAP kinase MAK-2 is an essential part of a signaling cascade involved in germling fusion (Pandey et al. 2004). To further characterize this signaling pathway we analyzed the role of BEM-1, a possible scaffold for MAK-2. The orthologous protein Bem1p in *Saccharomyces cerevisiae* is involved in polarity establishment during budding and shmoo formation. In contrast, a *N. crassa bem*-1 knock out mutant is not significantly impaired in spore germination and polar hyphal growth. However, *bem*-1 germlings are fusion defective and exhibit no chemotropic interaction. We determined the subcellular localization of BEM-1 by using GFP fusion constructs. We detected BEM-1-GFP accumulation at growing hyphal tips similar to previously reported data from *Aspergillus nidulans* (Leeder and Turner, 2008). In addition we found two more regions of BEM-1-GFP accumulation. In germling fusion pairs, BEM-1-GFP localizes to the tips of both partners and concentrates at the fusion point, once cell-cell contact has been established. Deconvolution microscopy and subsequent 3-D reconstruction revealed BEM-1-GFP localization around the opening fusion pore, detectable as a bright ring. We also detected BEM-1-GFP at septa of germ tubes and mature hyphae. Taken together, our data suggest novel functions of BEM-1 in chemotropic growth, fusion pore formation and septa formation or maintenance. In our further studies we will try to unravel and identify the distinct molecular functions of BEM-1 during *Neurospora* development. (Poster # 311)

The structure of organelles and the distribution of calcium transporters in *Neurospora crassa*.

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Calcium is an important signaling molecule, hypothesized to play a major role in polar growth of fungal hyphae. Analysis of the genome indicates *N. crassa* has genes for at least 5 major calcium transporters. These are an H+/Ca++ exchanger, named *cax*, and 4 Ca^{++}-pumping ATPases, named *nca-1, nca-2, nca- 3*, and *pmr*. We are investigating the role of these proteins in regulating intracellular levels of calcium. The phenotypes of mutant strains lacking transporters have been characterized and each has been tagged with GFP and/or dsRED. Because the structure and abundance of organelles is poorly described in *N. crassa* we examined the location of organellar marker proteins tagged with GFP and dsRED. The results show that the organelles, especially the vacuole, have different structure and abundance in different regions of the hypha. The large, fast growing hyphae of *N. crassa* permit a striking visualization of intracellular structure. Ca-transporters appear in the plasma membrane, the vacuole, the Golgi and the ER/nuclear envelope. Near the hyphal tip we also observed an organelle not previously described that is made visible with tagged vacuolar ATPase or the *cax* transporter. (Poster # 273)

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Two Golgi apparatus COG proteins are important to polarity establishment and maintenance in *Aspergillus nidulans*.

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*swoP*1 (swollen cell) and *podB*1 (polarity defective) mutations in *Aspergillus nidulans* interfere with establishment and maintenance of polarity. At restrictive temperatures, conidia of *swoP*1 may swell to approximately 1.5 times the normal diameter, produce abnormally wide hyphae and/or establish multiple points of polarity, which grow isotropically before arrest. Conidia of *podB*1 never establish polarity at restrictive temperatures. Cell walls of both strains are as thick as 1 µm (TEM) compared to ca. 0.04 µm at 28C, and the cytoplasm contains numerous irregular membrane structures. Genes complementing the mutations of *swoP*1 and *podB*1 have strong sequence homology to COG4 (AN7462) and COG2 (AN8226), respectively. Sequencing of the respective loci reveals point mutations causing truncations near the C-terminus. In mammals and yeast, COG2 and COG4 are part of a multi-protein structure called the COG (conserved oligomeric Golgi) complex associated with retrograde transport within the Golgi apparatus. To provide evidence for a COG function of AN7462 and AN8226, we used a high-copy AMA1 plasmid to overexpress the COG homologues of *A. nidulans* COG1-4, COG6, COG7, as well as the functionally-related homologues YPT1 (Rab GTPase) and HOC1 (mannosyl transferase). High copy expression of COG2 corrected the *swoP*1 phenotype while the remaining proteins did not. High copy expression of COG3 and COG4 corrected the *podB*1 phenotype while the remaining proteins did not. Collectively, these results support a conclusion that the SwoP and PodB proteins function in a common complex including a predicted COG3, which is consistent with the structure of the "A lobe" of yeast and mammalian COG models. (Poster # 263)

Actin binding proteins and endocytosis during tip growth of *Neurospora crassa*

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Filamentous fungi are ideal models to study the processes that determine polarized growth. Actin is important for both polarized hyphal growth and the Spitzenkörper (Spk) function. Coronin is an actin binding protein that plays a major role in regulating actin organization and cell locomotion and growth in eukaryotic cells. To determine the role of coronin in polarized hyphal growth and Spk function in *Neurospora crassa*, we have studied the cellular features of a coronin homolog (*cor1*) mutant and compare it with the wild type (WT) strain. Disruption of *cor1* resulted in delayed polarity establishment during spore germination, reduced growth rates, distorted hyphal morphology and growth patterns and a branching rate that was fivefold higher than the WT. The Spk in *cor1* mutant was reduced in size and was altered in its morphology. Mitochondria and nuclei morphology and position were affected. Uptake of FM4-64 was delayed in the coronin mutant suggesting a disruption in the endocytotic process. The mutation of *cor1* gene in *N. crassa* is not lethal but negatively influences many cellular functions involved in hyphal growth and cytoplasmic organization. These observations support the vital roles that actin play in the cell and suggest that coronin is involved in multiple actin-regulated functions.

Circadian rhythms and photobiology: Sue Crosthwaite and Christian Heintzen

Coupling photochemistry to conformational change in fungal light sensors

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The PAS-LOV domain is a widespread signal transducing component that senses stimuli such as *l*ight, *o*xygen and *v*oltage. White collar 1 (WC-1) and Vivid (VVD) are two proteins with homologous LOV domains that entrain and gate circadian rhythms of the filamentous fungi *Neurospora crassa* in response to blue light. Through structural and biochemical studies we have probed the molecular mechanisms that couple photon absorption to the conformational state of VVD. Light excitation of the FAD chromophore produces a cysteinyl-flavin adduct that restructures the protein and ultimately leads to dimerization. Photoinduced changes in flavin protonation state propagate to N-terminal regions of the protein, which change conformation to reveal new surfaces for mediating association. In general, LOV domains show dramatic variation in output signal and the lifetime of the photo-adduct signaling state. Mechanistic studies of slow-cycling VVD reveal the importance of reactive cysteine conformation, flavin electronic environment and solvent accessibility for adduct scission and thermal reversion. Specific active-site residues are key chemical determinants for deactivation and their substitution tunes photo-adduct lifetimes over four orders of magnitude. Overall, the current data provides a comprehensive model for how fungal light sensors switch between inactive and active states.

Light sensing in *Aspergillus nidulans*

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Light sensing is a common feature of many filamentous fungi. They use this parameter to orient in the environment, trigger developmental decisions or metabolic capacities. Several putative light receptors can be found in all fungal genomes although detailed studies are limited to a few species. In *Aspergillus nidulans* we showed that phytochrome (FphA) is part of a protein complex containing the white-collar homologues LreA and LreB, the two central components of the *Neurospora crassa* blue-light sensing system. We found that FphA represses sexual development and mycotoxin formation, whereas LreA and LreB stimulate both. Surprisingly, FphA also interacted with VeA, another regulator involved in light sensing and mycotoxin biosynthesis. All protein interactions occurred in the nucleus, despite cytoplasmic subfractions of the proteins. According to the developmental effects we asked whether there are phase-specific transcription factors that are part of the light regulation complex (LRC) or interact with it. We tested a number of candidate proteins and found that NosA (= number of sexual spores) and NsdD (= never sexual development) are part of the LRC. The interaction between NosA and phytochrome was restricted to the nucleus, whereas NsdD and FphA interacted in the cytoplasm. We also analyzed the binding of the light regulator complex to different promoters of putative light regulated genes. Chromatin immuno-precipitation experiments revealed that FphA binds to *ccgA* (*N. crassa ccg-1*) and conJ (*N. crassa con-10*). These results suggest that the transmission of the light signal possibly occurs at the promoters of light-dependent genes.

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Photoreceptor genes in *Phycomyces*

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The zygomycete *Phycomyces blakesleeanus* responses to light include phototropism of the fruiting body, induction of beta-carotene biosynthesis, and regulation of fruit body development. All of the responses to light require the products of the madA and madB genes. madA encodes a protein similar to the Neurospora blue-light photoreceptor, zinc-finger protein WC-1, and madB encodes a protein similar to the Neurospora zinc-finger protein WC-2. MADA and MADB interact to form a complex in yeast two-hybrid assays and after co-expression in E. coli, suggesting that the responses to light are mediated by a photoresponsive transcription factor complex. The Phycomyces genome contains three wc 1 genes and four wc 2 genes, including madA and madB. In addition, the genome contains a gene, cryA, similar to the blue-light photoreceptor cryptochrome DASH. Most of the photoreceptor genes are induced by light, except madA and madB. wcoA, wctB, and wctD are induced by low-intensity light , while wcoB and cryA need high-intensity light for photoinduction. Gene photoinduction is impaired in madA and madB mutants. The presence of multiple wc genes may allow perception across a broad range of light intensities, and formation of specialized photoreceptors for particular photoresponses.

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Circadian Clock Output Pathways in *Neurospora crassa*

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Organisms from bacteria to humans use a circadian clock to control daily biochemical, physiological, and behavioral rhythms. We are using the simple model organism *Neurospora crassa* to study the clock and its output pathways.
Using microarrays we found that about 20% of Neurospora genes are under control of the circadian clock system at the level of transcript accumulation, and that the bulk of the clock-controlled mRNAs have peak accumulation in the late night to early morning. These data suggest the existence of global mechanisms for rhythmic control of gene expression. Consistent with this idea, we found that the Neurospora OS pathway, a phosphorelay signal transduction pathway that responds to changes in osmotic stress, functions as an output pathway from the FRQ/WCC. ChIP/Solexa sequencing with known oscillator proteins revealed that phosophorelay/MAPK pathway components are direct targets of the White Colar Complex (WCC), providing a direct connection between the clock and the output pathway. Activation of the OS pathway by the FRQ/WCC oscillator culminates in rhythmic OS-2 MAPK activity, which through time-of-day-specific activation of downstream effector molecules, controls rhythms in several target clock-controlled genes. Hijacking conserved signaling pathways by the circadian clock provides a new paradigm for global rhythmic control of target genes of the pathway.

Chromatin-remodeling and epigenetics at the *frequency* locus

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Circadian-regulated gene expression is controlled by a transcriptional negative feedback loop and a recent body of evidence indicates that epigenetics and chromatin- remodeling are integral to this process. We have previously determined that multiple ATP-dependent chromatin-remodeling enzymes function at the clock gene *frequency (frq)*. We now report that the Neurospora homologue of a chromodomain helicase DNA-binding (*chd2*) gene is required for epigenetic transfer of time and remodels chromatin at the *frq* locus. Specifically, there seems to be a major defect in remodeling at the *frq* antisense promoter in the *Delta-chd2* strain. Unexpectedly, our studies on CHD2 activity revealed that DNA sequences within the *frq* promoter are typically methylated and are hypermethylated in *Delta-chd2* strains. Normal WT DNA methylation requires both a functional circadian clock as well as the *frq* antisense transcript, *qrf*, indicating that clock components contribute to the regulation of DNA methylation. Furthermore, the DNA methyltransferase, DIM-2, is required for *frq* methylation. Phenotypic characterization of *Delta-dim-2* strains shows that DNA methylation is not necessary for rhythms, although these strains display a minor phase advance of approximately 2 hours suggesting that promoter methylation may be used to fine tune gene expression. (Poster # 119)

Dynamics of nuclear localization *in vivo* of the *frq* gene product in *Neurospora crassa* during the circadian rhythm

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The circadian rhythm of *Neurospora crassa* is expressed on the surface of an agar medium as alternating bands of conidiating regions from actively growing cultures. Almost all of the molecular information about this rhythm has been obtained in underwater disk shaking cultures that are not actively growing. To reconcile this two drastically different culture conditions, a new set of tools have been developed, ie the use of a red fluorescent protein (RFP) as a reporter in combination with time-lapse confocal microscopy. This reporter was codon optimized for expression in *Neurospora* and was named mCherryNC. The expression of mCherryNC driven by the *ccg-2* (clock- controlled gene) promoter was examined in vegetative hyphae of growing cultures under fluorescence and confocal microscopy. RFP showed temporal expression, reflecting the circadian control of the *ccg-2* gene and spatial expression, ie it was localized to aerial hyphae and conidia. The mCherry reporter was also fused to the C-terminal end of the FRQ (frequency) protein and examined during a complete circadian rhythm under the control of the *ccg-2* and *frq* promoters. The fluorescence of FRQ-mCherry, driven by either promoter, was detected only at the growing edge of the colony; it was observed both in the cytoplasm and nuclei of vegetative hyphae for a distance of approximately 150-200 µm from the cell apex. Accumulation of FRQ-mCh in nuclei under the control of the *ccg-2* or *frq* promoters showed two peaks of fluorescence. Clearly, the RFP mCherryNC proved to be a new and useful tool to monitor the circadian rhythm at the cellular level in growing cultures of *N. crassa*. (Poster # 297)

Posttranscriptional regulation of the Neurospora circadian clock

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The eukaryotic circadian oscillators consist of autoregulatory transcription-based negative feedback loops. However, the role of post-transcriptional regulation in circadian oscillators is unclear. In the Neurospora circadian clock, FREQUNENCY (FRQ) and a FRQ-interacting RNA helicase (FRH), form the FFC complex that represses the transcription of frq to close the circadian negative feedback loop. Here we show that, in addition to its role in transcriptional repression, FFC binds frq RNA and interacts with the exosome and to post-transcriptionally regulate frq decay. Consequently, frq RNA is robustly rhythmic as frq is more stable when FRQ levels are low and less stable when FRQ levels are low. Knock-down of RRP44, the catalytic subunit of the exosome, elevates frq RNA levels and impairs clock function. In addition, rrp44 is a clock-controlled gene and a direct target of the WHITE COLLAR complex. Our results further show that RRP44 is required for the circadian expression of a subset of ccgs. Taken together, these results suggest that FFC and the exosome are part of the post-transcriptional circadian negative feedback loop regulating frq levels. And as the machinery for mRNA degradation, exosome defines a novel post-transcriptional negative feedback loop in circadian system. (Poster # 168)

Cycling without cyclins: a transcription network oscillator.

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Early work in frog and marine invertebrate embryos suggested that cell-cycle events are controlled by a biochemical oscillator centered on the cyclin dependent kinase (CDK) complex. Accordingly, *S. cerevisiae* cells lacking all of the S-phase and mitotic cyclin genes, cannot replicate DNA or enter mitosis. However, these cyclin mutant cells continue to exhibit cyclic behaviors with a period very similar to a normal cell cycle. In a genome-wide study of transcript dynamics in cyclin mutant cells, we found that ~70% of genes that are normally expressed periodically in wild-type cells continue to be expressed on schedule in these cyclin mutant cells. We constructed a transcription factor network model from our data that could explain the behaviors we observed. Dynamical models indicate that the transcription factor network could function as a robust cell-cycle oscillator independent of S-phase and mitotic CDK activity. We have perturbed the expression of transcription factors within the network and observed significant changes in the oscillatory period of cyclin mutant cells. These findings suggest that the transcription factor network may function as an oscillator that controls cell-cycle period. Further experiments that examine the coupling of the transcription factor network oscillator to other known cell-cycle regulatory mechanisms will be discussed. Our findings support a model in which a transcription factor network oscillator cooperates with CDK activity and checkpoint mechanisms to produce robust cell-cycle oscillations.

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Basidiomycete biology and genomics: Sarah Watkinson and Dan Cullen

Laccase and other multi-copper oxidase genes in Agaricomycotina

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Laccases as redox enzymes oxidize various phenolic compounds. Fungal laccases are thought to play roles in substrate degradation, in developmental processes and in stress reactions. Genomes of higher basidiomycetes offered the possibility to detect laccase genes by in silico methods. Surprisingly, higher basidiomycetes can have large families of laccase genes – for example, *Coprinopsis cinerea* has 17 different ones. In addition to laccase genes, other species have genes for related multi-copper-enzymes with poorly or not defined properties. Here, I present data on evolution of the families of laccase and other multi-copper enzymes. From species comparison it appears that genes duplicated late in evolution. Analysis of alleles of laccase genes in different strains of *C. cinerea* suggest that at least some of the duplicated genes can be lost without any problem. Data on gene expression through the fungal life-cycle will be presented as well as data on protein identification. Transformants of laccase genes under the control of the *gpdII* of *Agaricus bisporus* are used to produce high amounts of enzyme for protein purification and characterisation. Proteins encoded by the different laccase genes of *C. cinerea* are found to differ in enzymatic properties.

Analysis of the *Postia placenta* genome reveals a novel paradigm for lignocellulose depolymerization.

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In contrast to white-rot fungi which degrade both lignin and polysaccharides in wood, brown-rot fungi hydrolyze only cellulose and hemicellulose, leaving behind a modified lignin residue. While the economic consequences of brown-rot wood decay are substantial, detailed knowledge of the enzymatic and genetic machinery of these organisms lags behind that of white rot fungi. The genome sequence of *Postia placenta*, a brown-rot basidiomycete fungus, was recently completed by the Joint Genome Institute and an international consortium of co-investigators. Examinations of the *P. placenta* genome, transcriptome, and secretome have revealed a unique constellation of extracellular enzymes, including an unusual repertoire of extracellular glycoside hydrolases. Genes encoding exocellobiohydrolases and cellulose-binding domains, typical of cellulolytic microbes, are absent in this fungus. When *P. placenta* was grown in medium containing cellulose as sole carbon source, transcripts corresponding to several hemicellulases and to a single putative beta-1-4 endoglucanase were expressed at high levels relative to glucose-grown cultures. Also upregulated during growth in cellulose medium were putative iron reductases, quinone reductase, and structurally divergent oxidases potentially involved in extracellular generation of Fe(II) and H_2O_2. These observations are consistent with a biodegradative role for Fenton chemistry in which Fe(II) and H_2O_2 react to form hydroxyl radicals, highly reactive oxidants that are capable of depolymerizing cellulose and hemicellulose. The availability of the *P. placenta* genome also offers insight into the phylogeny of lignocellulose- degrading fungi. For example, comparisons between *P. placenta* and the closely related white rot fungus, *Phanerochaete chrysosporium*, support an evolutionary shift from white rot to brown rot, during which genes and gene families were lost, including those encoding lignin peroxidases and manganese peroxidases which are required for depolymerization of lignin. (Poster # 100)

Recent updates on the genome project of the mycorrhizal basidiomycete *Laccaria bicolor*

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Laccaria bicolor is a telluric basidiomycete ubiquitous to diverse forest ecosystems. This biotroph fungus is intimately associated to tree roots to form the ectomycorrhiza, a specific symbiotic organ. This fungus has a beneficial impact on tree growth and health and thus plays a key role in the functionning of the forest ecosystem. The sequencing of the genome of *L. bicolor* has paved the way to the comprehension of the complex mechanisms underlying this tree/fungus interaction. The 60Mb of this fungal genome contains about 19,000 predicted genes and a large catalog of transposable elements and repeated sequences. One year after the publication of the high-quality draft and annotation of the genome, we will present an update on the sequence finishing and the most recent gene analysis based on proteomics and transcriptomics. The later revealed an expanded set of mycorrhiza-induced small secreted proteins (MISSP) that are specifically expressed during the interaction with host plants. Immunocytolocalisation has confirmed their accumulation in the symbiotic tissues. RNAi-based gene inactivation is currently underway to unravel the role of *L. bicolor* MISSP.

Environmental regulation and transcript profiling of the reproductive phase change in *Agaricus bisporus*

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We have investigated the phase change from vegetative mycelium to reproductive growth in the white cultivated mushroom *Agaricus bisporus*. Environmental triggers are known to regulate fruiting in higher fungi and this knowledge has been used by the mushroom cultivation industry to control crop production. Temperature, carbon dioxide level and the concentration of 8-carbon volatiles are involved in the reproductive phase change in *A. bisporus*. However, it is not known how each stimulus affects the process at the molecular level and whether the control of fruiting is under a single or sequential regulatory mechanism. A suppression subtractive hybridisation and custom oligo-microarray screening approach was employed to investigate the transcriptional changes in *A. bisporus* to environmental and morphogenetic change. The initiation of fruiting under standard growing conditions was compared with experiments where temperature, carbon dioxide or volatile levels were not altered. The data has been used to profile the response of *A. bisporus* genes during fruiting and to identify the genetic response to individual stimuli. Evidence shows temperature affects later stages of mushroom development, while carbon dioxide and volatile levels regulate the initial switch from vegetative mycelium to reproductive phase. (Poster # 154)

Ribosomal synthesis of amatoxins in Amanita and Galerina

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Genome survey sequencing of *Amanita bisporigera* revealed that the cyclic peptide amatoxins and phallotoxins are encoded ribosomally as part of a proprotein. The eight and seven amino acids, respectively, of amatoxins and phallotoxins are flanked by conserved N-terminal and C-terminal regions, which are also found in many uncharacterized *Amanita* peptides (identified by genome survey sequences, PCR and reverse transcript PCR, and Southern blotting). *Galerina marginata*, another amatoxin-producing agaric, possesses similar machinery, albeit the conserved regions differ sufficiently to preclude cross-hybridization of amatoxin gene family probes between genera. We consider the possibility of a wider application of this type of "combinatorial peptide factory" in basidiomycetes, particularly given the relative lack of non-ribosomal peptide synthetases in these fungi.

Comparative genomics of basidiomycete peptidases.

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BlastP searches of the gene prediction sets of eight sequenced haploid basidiomycetes against the MEROPS Peptidase Database, followed by manual annotation, demonstrated that peptidase genes comprise 1.3 % (*L. bicolor* to 2.9 % (*U. maydis* of basidiomycete genomes. The major mechanistic families of peptidases (aspartic, cysteine, metallo, serine, and threonine) are represented to different degrees in each genome. Eighty different MEROPS peptidase families are represented over the eight species. Only seven families have a single gene in each organism. Metallopeptidases and serine peptidases each represent about 1/3 of the total peptidase complement. A hallmark of basidiomycete peptidases is the large expansion of some gene families. The family showing the greatest expansion across the genomes is the aspartic peptidase family A1, with gene numbers ranging from 7 in *C. neoformans* to 49 in *P. chrysosporium*. Trees of similarity suggest that some gene expansions pre-date speciation events, while others are more recent. The former is clearly seen with the evolution of the 14 T1 threonine peptidases, which are components of the proteasome. The latter can be observed in multiple instances including the *C. cinerea* 27 member M43B family, where clear evidence of gene duplication is seen in the 12 genes located on chromosome 10. Current microarray evidence in *C. cinerea* indicates that members of expanded peptidase families are not coordinately regulated. This, along with evidence for positive selection between certain family members, implies that individual peptidases within some families may have novel functions.

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Population genomics in the forest pathogen *Heterobasidion annosum*.

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Heterobasdion annosum s.s., a pathogenic basidiomycete causing root and butt rot to conifers, is found across Europe. It is part of the *H. annosum s.l.* species complex, with members in both Europe and North America and host specificities towards different conifers. The pathogenic lifestyle as well as the possibility to obtain monokaryotic isolates makes this species interesting and suitable for population genomic analysis. Recent advances in sequencing technologies have enabled the move from population genetics to population genomics. We have sequenced the genomes of several individuals of *H. annosum s.s.* using the sequencing by synthesis Illumina/Solexa platform. We have used *de novo* assembly as well as mapping of sequencing reads to the genome of a closely related *H. annosum* species to reconstruct the genomes of the sequenced individuals. This allows us to describe the genomic variation between the individuals within the focal species. Single nucleotide polymorphism (SNP) mining in the Solexa reads provided more than 100 000 SNPs. On a whole genome scale, we address questions of population structure, linkage disequilibrium and recent selective events. (Poster # 435)

Deep Rot: phylogenetics and comparative genomics of brown-rot basidiomycetes.

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Lignocellulose in woody plant cell walls is a major repository of organic carbon. Basidiomycetes are the major group of microorganisms that are able to degrade this resource, making them important actors in the global carbon cycle. Two modes of wood decay have been described in Basidiomycetes, white rot, in which both lignin and cellulose are degraded, and brown rot, in which cellulose is depolymerized without extensive lignin degradation. It has been suggested that brown rot is an efficient mechanism that allows Basidiomycetes to access cellulose without the energetically costly process of lignin decay. We are studying the evolution of decay mechanisms in basidiomycetes using molecular phylogenetics, molecular clock analyses, and comparative genomics. Molecular phylogenetic analyses indicate that the brown rot strategy has evolved several times in the Basidiomycetes, and may be correlated with shifts to specialization for conifer substrates. Molecular clock analyses suggest that the independent origins of brown rot occurred in overlapping time periods, well after the diversification of both angiosperms and conifers. The recently completed genome of the brown rot polypore *Postia placenta* complements that of the white rot fungus *Phanerochaete chrysosporium*, and provides opportunities to assess the genetic bases of the transition to brown rot. There have been multiple independent losses of cellulolytic enzymes in the lineage leading to Postia, as well as losses of class 2 fungal peroxidases, which are thought to function in lignin degradation. These findings are consistent with the view that the transition to brown rot is associated with reduction of the energetically expensive mechanism of white rot. We are now studying the genome of *Serpula lacrymans*, which represents an independent origin of brown rot in basidiomycetes.

CONCURRENT SESSIONS II

RNA functions, Epigenetics and Chromosome Biology: Hitoshi Nakayashiki and Yi Liu

How is RNA silencing invoked against the retrotransposon MAGGY in *Magnaporthe oryzae*?

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Recent large scale sequencings of small RNAs in various eukaryotic organisms have revealed that the major endogenous targets of RNA silencing are repetitive elements, especially retrotransposons. However, the molecular mechanisms underlying how RNA silencing is specifically triggered against transposable elements are poorly understood. MAGGY is a Ty3/gypsy-like LTR-retrotransposon found in the rice blast fungus *Magnaporthe oryzae*. This element was targeted for RNA silencing and DNA methylation in the original host and even in MAGGY-free strains of *M. oryzae* when introduced by transformation. To gain insight into the molecular mechanisms leading to the initiation of RNA silencing against retrotransposons, we constructed a series of MAGGY mutants. We then examined their ability to trigger RNA silencing in a MAGGY-free isolate. Deletion and frameshift mutation in gag, reverse transcriptase and integrase domains abolished transpositional activity of the element. Interestingly, MAGGY-related siRNA accumulation was drastically reduced in the transposition deficient mutants. The level of siRNA accumulation was associated with the type of mutant rather than the copy number of the mutant in the genome. Analysis of site-directed mutants of MAGGY genes suggested that siRNA biogenesis was induced in some stages of retrotransposition later than reverse transcription. A possible model for invoking RNA silencing against the MAGGY element in *M. oryzae* is discussed.

qiRNA, a novel type of small inteference RNA induced by DNA damage

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RNA interference (RNAi) is a conserved eukaryotic gene regulation mechanism that uses small interfering RNAs to trigger posttranscriptional or transcriptional gene silencing. In addition to the microRNAs, recent studies have uncovered the existence of several types of endogenous small RNAs (20-30 nt) in animals and plants, including endogenous siRNAs, Piwi-interacting RNAs (piRNAs), rasiRNAs and 21U RNAs. The evolutionary origin of those small RNAs are pooly understood. Here we report a novel species of small RNA induced by DNA damage in *Neurospora.* The biogenesis of these small RNA requires several components of the quelling pathway, which is a posttranscriptional silencing mechanism in *Neurospora*. The function of this type of small RNAs and their relationship to other eukaryotic small RNAs will be discussed.

The gene silencing mechanism in *Mucor*: consensus and dissent

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RNA silencing is a conserved regulatory mechanism that has challenged the central dogma of molecular biology and highlighted the regulatory role of small RNA molecules. Although the machinery of RNA silencing is well conserved throughout the evolutionary scale, the number of paralogous silencing proteins differs considerably among species, as it does the specificity of each protein in different RNA silencing pathways. We have used the zygomycete *Mucor circinelloides* as a model organism to investigate the silencing mechanism in this class of fungi. Gene silencing in *Mucor*, contrary to what happens in most of Ascomycetes, is induced by non-integrative sense or inverted-repeat transgenes and it is associated with the accumulation of two size classes of antisense siRNA, 21-nt and 25-nt long, which are differentially accumulated during the vegetative growth. We have cloned and functionally characterized several genes belonging to the main gene families involved in the silencing machinery (*dicer*, *argonaute*, *rdrp* genes). Phenotypic and molecular analysis of null mutants affected in those genes highlights the peculiarities of the gene silencing mechanism in *Mucor*, and suggests a role of the RNA silencing pathways in the control of endogenous functions.

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Small RNA Pathways in the Oomycetes *Phytophthora sojae*, *Phytophthora ramorum*, and *Phytophthora infestans*.
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The oomycetes are a distinct lineage of eukaryotes that contain many important pathogens of plants, including *Hyaloperonospora parasitica* and several *Phytophthora* species. Plant pathogens of the genus *Phytophthora* are among the most well-studied oomycetes and include *P. infestans*, a pathogen of potato that caused the Irish potato famine, *P. sojae*, a pathogen of soybean, *P. ramorum*, the cause of sudden oak death, and *P. capsici*, a pathogen of pepper. Recently the genomes of these four *Phytophthora* species and *H. parasitica* were sequenced. Most eukaryotes have RNA silencing systems that use small RNAs to suppress a wide range of genes, genetic elements, and viruses. One important silencing pathway is the microRNA pathway. miRNAs (usually 21-22 nucleotides) are derived from processing of self-complementary foldback RNAs derived from *MIRNA* genes. miRNAs associate with protein effector complexes containing ARGONAUTE proteins, and the miRNA serves to guide cleavage, translational repression or redirection of the target transcript within the cell. Here, we identified the core small RNA biogenesis components and effectors in three *Phytophthora* species, *P. sojae*, *P. ramorum*, and *P. infestans*. We used high-throughput pyrosequencing (454 Life Sciences) and sequencing-by-synthesis (Illumina) to profile small RNA. Analysis of these data revealed several candidate *MIRNA* genes from one gene family that are conserved in all three species. In addition, large numbers of siRNA-generating loci were identified throughout the *Phytophthora* genomes. (Poster # 83)

Chromosomal landscapes and secondary metabolite cluster expression. Nancy Keller, University of Wisconsin

Aspergilli are ubiquitous filamentous fungi whose members include human and plant pathogens and industrial fungi with tremendous medical, agricultural and biotechnological importance. Although demonstrating synteny along large tracks of their sequenced genomes, members of this genus vary remarkably in their secondary metabolome, possibly a reflection of a chemical arsenal important in niche securement. The sheer numbers of unique secondary metabolite (SM) genes highlight the genus as a potentially rich source of bioactive metabolites for medicinal and pharmaceutical use. Gene wealth, however, has not translated well into compound production, in part due to an inability to find conditions promoting expression of SM gene clusters. Here we demonstrate chromatin-level regulation of *Aspergillus* SM clusters suggestive of a role for epigenetic and telomere position effect mechanisms of cluster expression.

DNA damage checkpoint genes of *Neurospora crassa* are required for normal cell growth and maintenance of chromosome integrity
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Genome integrity is maintained by many cellular mechanisms in eukaryotes. One such mechanism functions during the cell cycle and is known as the DNA damage checkpoint. In *Neurospora crassa*, *mus-9* and *mus-21* are homologues of two key factors of the mammalian DNA damage checkpoint, ATR and ATM, respectively. The *mus-9* and *mus-21* mutants are sensitive to DNA damage and each mutant shows a characteristic growth defect: conidia from the *mus-9* mutant have a reduced viability and the *mus-21* mutant exhibits slow hyphal growth. Furthermore, strains carrying null alleles of both *mus-9* and *mus-21* could not be obtained. To facilitate the analysis of a strain deficient for both *mus-9* and *mus-21*, we first introduced a specific mutation to the kinase domain of MUS-9 to generate a temperature-sensitive *mus-9* allele (*mus-9^{ts}*) and then we crossed this strain to a *mus-21* mutant. Growth of the *mus-9^{ts} mus-21* double mutant ceased when switched to the restrictive temperature (37°C). Even at the permissive temperature (25°C), this strain exhibited a higher mutagen sensitivity compared to the *mus-9* and *mus-21* single mutants, as well as slow hyphal growth and low viability of conidia. Interestingly, we also observed the accumulation of micronuclei in the conidia of this double mutant, which may indicate fragmentation or missegregation of chromosomes. Based on these data, we suggest that *mus-9* and *mus-21* have redundant roles in the DNA damage response and the maintenance of chromosome integrity.

Centromeric regions of *Neurospora crassa* are composed of heterochromatin.
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Centromeres serve as platforms for the assembly of kinetochores and are essential for nuclear division. While pericentric heterochromatin has been documented in many eukaryotes, recent studies suggest that centromeric core chromatin is free from silencing modifications. We generated GFP and FLAG fusions to the centromere proteins CenH3 (CENP-A) and CKP-1 (CENP-C) and identified Neurospora centromeres by ChIP- sequencing. On each chromosome we found a ~100 to 200 kb region that overlaps with centromeric regions previously predicted by genetic mapping, DNA composition and presence of transposon relics. We asked which histone modifications are required for normal centromere function in Neurospora. Surprisingly, we found extensive co- localization of CenH3, CKP-1 and histone H3 K9 trimethylation (H3K9^{Me3}). In contrast, H3K4^{Me2}, which has been found at the cores of plant, fission yeast, Drosophila and mammalian centromeres, was not enriched at Neurospora centromeres. DNA methylation, which typically overlaps with Neurospora H3K9^{Me3}, was most pronounced at the centromere periphery but otherwise overlapped little with the distribution of centromere proteins. Mutation of dim-5, which encodes the predominant H3 K9 methyltransferase, resulted in loss of H3K9^{Me3} from the centromeres. By region-specific ChIP-PCR we observed concomitant loss of CenH3-GFP binding from all but the core of two centromeres that we investigated in greater detail. Similarly, CenH3-GFP was retained only at the core in the absence of HP1, the chromodomain protein that binds to H3K9^{Me3}. We show that eukaryotes make use of different strategies to maintain centromeres and suggest a model in which centromere proteins nucleate at the core but require DIM-5 and HP1 for spreading and maintenance of a normal centromere. (Poster # 266)

Substitutions in amino terminal tail of histone H3 cause dominant loss of DNA methylation in Neurospora

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In Neurospora DNA methylation depends on methylation of histone H3 lysine 9 (H3K9me) by the SET domain methyltransferase, DIM-5. Heterochromatin protein HP1 reads the trimethyl mark and directly recruits the DNA methyltransferase DIM-2 to regions destined for DNA methylation. Here we elucidate the role various amino acid residues of H3 on methylation of H3K9 and DNA. A single gene codes for histone H3 in Neurospora (*hH3*). We noted earlier that introduction of an ectopic H3 gene bearing a substitution at K9 caused dominant global loss of DNA methylation and reactivation of a silenced selectable marker (*hph*). We observed similar loss of DNA methylation for other alleles including those encoding R2L, A7M, R8A, S10A, T11A, G12P, G13M, K14Q and K14R. Except for R2L, K14Q and K14R these substitutions also resulted in a loss of DIM-5 activity *in-vitro*. In addition, we show that DIM-5 is sensitive to methylation of H3K4 and phosphorylation of H3S10. To test recessive effects of histone mutations, we used a null allele of histone H3 generated using RIP ($hH3^{RIP1}$) to create Neurospora strains that only have the desired substitution. The inability to isolate viable strains with only mutant version for some suggests that the corresponding residues are essential. In summary, we will present results suggesting that the amino terminal tail of histone H3 acts as a platform to integrate various signals to influence the methylation of H3K9 by DIM-5 and the ability of HP1 to read this trimethyl mark in order to regulate DNA methylation. (Poster # 114)

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Associations with plants: Martijn Rep and Erika Kothe

***Botrytis cinerea*; special aspects of a necrotrophic life style**

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Concerted efforts of the rapidly growing Botrytis community have made *B. cinerea* one of the best-investigated fungal pathogens; it is becoming a model system for the study of necrotrophy. Availability of molecular methods and the genome project speeded up functional analyses in the last years. A large set of knock out mutants and the increasing use of "omics" approaches helped to improve our understanding of the complex and highly variable life-style of this pathogen; still our knowledge is limited. I will briefly discuss the results of molecular analyses of "classical" pathogenicity determinants, i.e. the candidate gene approach; only very few true pathogenicity factors have been identified/confirmed so far by this approach. A promising strategy for the identification of new pathogenicity determinants is the use of mutants lacking signalling components/ transcription factors with central impact on specific steps in early pathogenesis, in combination with transcriptome analyses.
A major topic will be the role of the NOX complex for the interaction, and the impact of cross-talk of major signaling cascades (focus will be on the stress-activated MAPkinase) on the early infection processes.

A novel extracellular siderophore from *Epichloë festucae* is essential for grass mutualism.

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Epichloë festucae, a symbiotic fungal endophyte of cool-season grasses produces a fusarinine-type extracellular siderophore of novel structure, which we have designated epichloënine and an intracellular siderophore, ferricrocin. Loss of epichloënine by targeted gene replacement of the non-ribosomal peptide synthetase, *sidN* radically alters the mutually beneficial association of endophyte and host grass to that of an antagonistic one. In contrast, the symbiotic interaction was not affected by the elimination of ferricrocin through the targeted deletion of the non-ribosomal peptide synthetase *sidC*. Plants infected with the *delta-sidN* mutants are stunted, have altered root architecture, and typically senesce prematurely. Siderophore absence causes malfunctioning of the characteristic synchronized growth of the fungus with its plant partner. Hyphal elongation is no longer restricted to intercalary extension within expanding leaf tissue, but occurs by on-going branching followed by tip growth. Transmission electron microscopy of *delta-sidN* plants also revealed abnormalities in the hyphal ultrastructure including diffuse cytoplasm and the appearance of numerous vesicles outside the plasmalemma. Regulation of the NADPH oxidase complex which catalyses production of reactive oxygen species and controls hyphal morphogenesis has been affected in the *delta-sidN* mutants. Affymetrix Gene Chips® have been employed to further investigate the molecular mechanisms controlling global regulation of iron as well as control of hyphal morphogenesis and growth in the symbiotum. The results suggest that in the absence of epichloënine, iron homeostasis in the whole symbiotum is disrupted. We hypothesise that competition for iron is a critical factor in controlling endophytic fungal growth and hence for mutualism with grasses.

On full blast: signals, sensors and stress

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Understanding the processes that drive rice blast fungus germination, signal exchange between rice and *Magnaporthe oryzae*, and trigger signal relay leading to appressorium formation and plant penetration will, respectively, inform the design of prophylactic, curative and eradicant crop protection strategies [1]. Recently, we demonstrated the involvement of one member of an ancient multi-gene family [23] of secreted cutinases, *CUT2* in such signal exchange: being involved in degradation of the rice cuticle and in sensing the hydrophobic surface of its host. In turn, these events drive appressorium differentiation resulting in infectious growth in rice and barley [45]. Lately, we have considered signal relay downstream from early perception. This talk will allude to the role of a wall-associated protein in germination and to a particular AGC kinase involved in signal relay, but with this data set firmly in the context of genetic /biochemical events known to drive germination and subsequent infection structure formation.

[1]Skamnioti P and Gurr SJ (2009)*Trends in Biotechnology* (March) [2]Skamnioti P, Furlong RF and Gurr SJ (2008)*New Phytologist* 180 3 711-721 [3]Skamnioti P, Furlong RF and Gurr SJ (2008)*Communicative & Integrative Biology* 1 2 196-198 [4]Skamnioti P and Gurr SJ (2007)*Plant Cell* 19 8, 2674-2689 [5]Skamnioti P and Gurr SJ (2008)*Plant Signaling & Behavior* 3 4, 248-250

How oomycete and fungal effectors enter host cells.

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Oomycete and fungal plant pathogens produce effector proteins to condition host tissue for susceptibility. Many of these proteins can enter into the cytoplasm of plant cells, where they interfere with plant defense signaling and may also be recognized by intracellular plant resistance gene products. We have shown previously that conserved RXLR-dEER motifs in the N-terminus of several oomycete effectors enable these proteins to enter plant cells in the absence of any pathogen machinery. However, identification of motifs that fungal effector proteins to enter host plant cells have so far eluded identification. We carried out extensive mutagenesis of the oomycete RXLR-dEER motifs to improve our understanding of the spectrum of amino sequences that can enable host cell entry. Using this information, we have identified variant RXLR sequences in the N-termini of many fungal effectors, and have shown that they can enable host cell entry. Furthermore, we have identified a family of receptor molecules in plant cells that bind both oomycete and fungal N-terminal domains. Both the strength of binding and the specificity of binding are affected by mutations in the RXLR motif. This family of receptor molecules is also conserved in animal cells, and we have shown that the PEXEL motif of *Plasmodium* effectors ($RXLX^{E}/_{Q}$) can also bind to these receptors. Thus effectors from three different kingdoms of eukaryotic pathogens have evolved, convergently, the ability to target a highly conserved receptor family in eukaryotic hosts. The nature and location of the receptors support the hypothesis that the effectors enter host cells via receptor-mediated endocytosis.

Physical-chemical plant-derived signals induce differentiation in *Ustilago maydis*

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Ustilago maydis is able to initiate pathogenic development after fusion of two haploid cells with different mating type. Cell-cell recognition is based on a pheromone/receptor system. The pheromone signal is transmitted via a MAP kinase module leading to the activation of the Prf1 transcription factor, the essential regulator of sexual and pathogenic development. On the maize leaf surface, the resulting dikaryon switches to filamentous growth, differentiates appressoria and penetrates the host. Here we report on the plant signals required for filament formation and appressorium development in *U. maydis*. With the help of a marker gene that is specifically expressed in the tip cell of hyphae forming appressoria, in vitro conditions were established for filamentation as well as appressorium differentiation. Using a solopathogenic strain that is able differentiate without a mating partner, we show that hydroxy-fatty acids stimulate filament formation. These filaments resemble conjugation tubes. We show that hydroxy-fatty acids stimulate the induction of pheromone genes and this signal then activates the MAP kinase module.The hxdroxy-fatty acid signal can be bypassed by genetically activating the downstream MAP kinase module. Hydrophobicity also induces filaments and these resemble the dikaryotic filaments formed on the plant surface. When both signals are combined, about 30 % of the filaments develop appressoria. These results show that the early phase of communication between *U. maydis* and its host plant maize involves two distinct stimuli.To obtain insight into perception and downstream signaling after exppsure to hydrophobicity and/or hydroxy- fatty acids we performed microarray experiments using the artificial system. These results will also be presented. (Poster # 474)

What makes a biotrophic fungus a plant-pathogen or a symbiont? Insights from transportome analysis.

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Poplar trees are common used for production of pulp and consolidated wood products. More recently, woody plants are of great interest for carbon sequestration, biofuels production, and bioremediation. *Populus* is able to interact with biotrophic fungus, such as the symbiotic (*Laccaria bicolor*) and pathogenic (*Melampsora larici- populina*) basidiomycetes. During a plant-biotrophic fungus interaction, there is a fine-tuned metabolic association between partners. In pathogenic interaction, unilateral links predominate, whereas in symbiosis exchanges are bi-directional. Membrane transporters are thus key molecular players. Genomes of symbiotic (*L.bicolor*) and pathogenic (*M. larici-populina*) basidiomycetes interacting with *Populus* are now available. We take advantage of these genomic resources in order to identify genetic potential for membrane-transporters (transportome) and to provide insights into pathogenicity/symbiosis mechanisms. Results from comparative genomics and micro-arrays expression analysis will be presented. (Poster # 443)

The immune response of brown algae against the basal oomycete pathogen *Eurychasma dicksonii*

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The oomycete pathogen *Eurychasma dicksonii* is both the most abundant eukaryotic pathogen of marine brown algae, and the most basal member of the oomycete lineage. Despite being an obligate biotroph, it has the largest reported host range among marine pathogens - infecting virtually every brown algal species tested so far. Remarkably, virtually nothing is known about many fundamental aspects of its pathogenicity, biology, epidemiology, and ecology. Due to its availability in culture and the recently-completed sequencing of the genome of one of its main brown algal hosts (*Ectocarpus siliculosus*), *Eurychasma* is a particularly attractive model to study oomycete infection strategies and algal defense mechanisms. The reaction of different algal strains against *Eurychasma* range from extreme susceptibility to complete resistance against infection, suggesting a genetic basis for disease resistance in algae. In all cases investigated, resistance is associated with the early death of the challenged algal cell, which prevents further spread of the disease. This holds true across eight species tested, suggesting that resistance-associated cell death might be a conserved immune mechanism of brown algae. We will report our progress on the molecular characterization of this response, such as the development of *in situ* labeling techniques or mining of the *Ectocarpus* genome for potential disease resistance genes. (Poster # 484)

The biotrophic interfacial complex and the secretion of effector proteins into host cells during rice blast disease.

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The hemibiotrophic fungus *Magnaporthe oryzae*, causing the globally important rice blast disease, grows inside living rice cells by producing specialized invasive hyphae (IH) that are encased in a plant-derived extra-invasive-hyphal membrane (EIHM). Little is known how the fungus secretes effector proteins and delivers them into host cytoplasm across the EIHM to control host cells for successful disease, or to trigger host resistance. Using live-cell imaging and correlative light and electron microscopy, we identified a novel structure, the Biotrophic Interfacial Complex (BIC) that is suggested to mediate effector secretion. Each time the fungus enters a living rice cell, the BIC forms at the tip of the filamentous hypha and then is repositioned to the side of the first IH cell that differentiates from this filamentous hypha. BICs are a highly localized structure that consists of a complex aggregation of membranes and vesicles. Several blast effector proteins including AVR-Pita1, PWL1, and PWL2 are secreted into BICs when expressed by the fungus as fusion proteins with different fluorescent proteins (EYFP, EGFP, or mRFP). AVR-Pita and PWL signal peptides alone are able to direct this localized secretion. This process involves a classical ER-secretory pathway, because addition of the tetrapeptide ER retrieval signal to the C-terminus of AVR-Pita signal peptide:EGFP resulted in ER retention of fusion protein. EGFP expressed with AVR-Pita promoter and secreted with a non-effector signal peptide (from cutinase) did not show preferential BIC localization, suggesting that effector protein signal peptides contain information that controls BIC localization. We hypothesize that effector proteins are secreted first into BICs and then into the host cytoplasm. (Poster # 535)

Natural products and small molecules: Vera Meyer and Ben Horwitz

Nonribosomal peptide synthetase metabolites and fungal development

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Small molecule secondary metabolites play diverse, but also fundamental, roles in their producers, in addition to better known roles as effectors of inter-organismal interactions. Some of these are crucial for basic biological processes, such as growth, surface properties, asexual/sexual development, and nutrient gathering (e.g. iron), demonstrating the their significance to survival and proliferation of the producing fungus. We have deleted each of the 12 *Cochliobolus heterostrophus* nonribosomal peptide synthetases genes (*NPS*) to ascertain the role of the corresponding peptide products. Of the 12, five (NPS2, NPS4, NPS6, NPS10, NPS12) are conserved to some extent in ascomycete genomes, whereas seven are highly discontinuously distributed. All strains have been screened for alterations in morphology, hydrophobicity, growth, virulence, asexual and sexual development, and for resistance to various stresses. To date, four of the conserved genes are the only ones that yield phenotypes in the corresponding mutant strains when deleted. The observed phenotypes are: reduced virulence to the host (*NPS6*), alteration in morphology of colonies (*NPS10*) and surface properties (*NPS4*), reduced asexual sporulation (*NPS6*), reduced sexual development (*NPS2*), hypersensitivity to various stresses (*NPS6, NPS10*), all fundamental activities. None appears to require the *Cochliobolus* ortholog of *Aspergillus LaeA* for function. Two of the five conserved genes encode NRPSs that biosynthesize siderophores. NPS6 biosynthesizes an extracellular siderophore required for virulence and for combating oxidative stress and is up regulated in low iron. NPS2 biosynthesizes an intracellular siderophore and is required for ascospore production in homozygous deletion crosses, but not for virulence. Double deletion of *NPS2* and *NPS6* enhances virulence reduction, sensitivity to oxidative stress, and the sexual development phenotypes. Expression of *NPS6*, *ABC6* (adjacent to *NPS6*) and *CAT1* (catalase), but not *NPS2*, is under the control of the GATA transcription factor SreA, which negatively regulates their expression in adequate iron.

Aflatoxin biosynthesis is correlated to peroxisome functionality, lipid metabolism and oxidative stress in *Aspergillus flavus*

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Oxidative stress is a trigger for different metabolic events in all organisms and occurs during oxidative processes in the cell such as peroxisomal beta-oxidation of fatty acids. To study whether peroxisome functionality, oxidative stress and oxylipins formation are related to aflatoxin biosynthesis in *A.flavus* a gene encoding for a virus (*Cymbidium ringspot virus*) protein, p33, which is able to induce peroxisome proliferation, was inserted in NRRL 3357 WT strain. The peroxisome hyperproliferation in Afp33 strains was demonstrated by TEM analysis. These organelles were also labelled with a SKL (target peptide for peroxisome) - dsRED fluorescent protein. The p33-GFP was found to co-localise with the red dsRED fluorescent protein. The expression of some peroxisome functionality markers (*foxA*, *pex11*) was also monitored. The WT and the mutant strains were compared by means of phenotype microarray technique. In Afp33-dsRED strain an up-regulation of the lipid metabolism (upregulation of the TCA cycle, FFA beta-oxidation and TG accumulation) is the putative cause for the induction of a hyperoxidant status (higher ROS and oxylipins formation) and aflatoxin biosynthesis enhancement both *in vitro* and *in vivo* (maize seeds). *In silico* N_SITE analysis of the *aflR* promoter region indicates the presence of regulatory elements (RE) responsive to CREB (cAMP), SREBP-ADD1 (lipid metabolism), AP1 (human ortholog of Yap1 and ApyapA), and PPARalfa (lipid metabolism) binding factors, further stressing a close connection among the onset of oxidative stress, oxylipin formation and toxin synthesis. (Poster # 239)

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Potent inducers of deoxynivalenol production by *Fusarium graminearum*

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Fusarium head blight of wheat, caused by *F. graminearum*, is one of the most important diseases of wheat not only because of yield losses but also the contamination of grain with trichothecene toxins such as deoxynivalenol (DON). As well as playing an important role in the virulence of the pathogen, DON has severe health consequences for humans and animals which consume contaminated grain. An intriguing aspect of the pathogen's biology is that the production of DON occurs at much higher levels during the infection process than during axenic culture, even on plant-derived media such as autoclaved grain. Presumably, the fungus produces toxins in response to unknown signals of plant origin. We developed a reporter strain of *F. graminearum* carrying a *TRI*-gene promoter linked to the green fluorescent protein gene and used this strain in a high-throughput screen to identify compounds that induce high levels of DON production in culture. Through this system, we have identified a number of compounds that induced the genes involved in the biosynthesis of DON to levels equivalent to those observed during infection, and resulted in extremely high concentrations (>1500 ppm) of DON being produced in culture filtrate after seven days of culture. Some of these compounds are naturally present in wheat and increase in concentration in heads following inoculation suggesting that they may act as *in planta* DON inducers. In addition, *F. graminearum* genes co-regulated under high DON-inducing culture conditions have been studied using Affymetrix expression profiling and other co-regulated fungal genes and gene clusters identified. Together, these studies open the way to reduce the production of DON during infection process by either genetic or chemical modulation of the production and/or action of host inducers. (Poster # 498)

***Stagonospora nodorum* utilizes multiple host-selective toxins which act as effectors of pathogenicity to induce disease on wheat**

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The *Stagonospora nodorum*– wheat interaction has been shown to involve multiple proteinaceous host-selective toxins (HSTs) that interact either directly or indirectly with dominant sensitivity gene products in wheat. Currently we have identified and published four HST–host gene interactions including SnToxA-*Tsn1*, SnTox1- *Snn1*, SnTox2-*Snn2*, and SnTox3-*Snn3*. In each case, toxin sensitivity is controlled by a single dominant gene. Using QTL analysis, significance of these interactions ranged from 18 to 63% of the disease variation, highlighting the importance of these interactions. Recently, two new HST-wheat sensitivity gene interactions have been characterized. A proteinaceous HST temporarily named SnTox4B was shown to be between 10 and 30 kDa and interact with the wheat gene *Snn1A* found on chromosome 1A. The SnTox1A-*Snn1A* interaction accounts for as much as 44% of the disease variation. Another proteinaceous HST temporarily named SnTox4B, also in the range of 10-30kDa interacts with a gene product identified on wheat chromosome 4B temporarily named *Snn4B*. The SnTox4B-*Snn4B* interaction has been identified in both hexaploid (bread wheat) and tetraploid (durum wheat) populations and has been shown to account for as much as 9% and 61% of the disease variability, respectively. To date we have accumulated solid evidence for six HST-host gene interactions as well as preliminary data for the presence of several additional significant interactions. This work establishes S. nodorum blotch as a model inverse gene-for-gene system where proteinaceous HSTs, acting as effectors of pathogenicity, interact with dominant host gene products resulting in a compatible disease interaction. (Poster # 551)

Screening of secondary metabolites important in fungal interactions: The challenge of data mining

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Fungi produce a vast diversity of chemical compounds. Secondary metabolites are especially important in the establishment and survival of these organisms in the environment. The purpose of this research is to identify and monitor secondary metabolites involved in the interaction of *Fusarium verticillioides* and *Ustilago maydis* in maize (*Zea mays*). A first step to achieving this goal is to screen for metabolites (i.e. fusaric acid, ustilagic acid, etc.) produced during *in vitro* interactions. We explore different strategies for data analysis in aims to answer three main questions: 1) What compounds (known and unknown) are produced by *F. verticillioides* and *U. maydis* in vitro?; 2) Is any compound up or down regulated as result of species' interactions?; 3) Is there any compounds produced *de novo* or completely suppressed during fungal interactions? Isolates of *F. verticillioides* (endophytic isolates) and *U. maydis* were grown separately and in combination in solid media. Co-inoculated cultures fully colonized the media at day ten, time point when agar plugs where extracted for metabolic analyses. An UPLC/TOF/MS (Ultra High Performance Liquid Chromatography/Time of Flight/Mass Spectrometry) instrument was used for data collection and data processing was done with the software MarkerLynks (Waters Corporation). *U. maydis* chromatograms where highly complex containing approximately 100 peaks while chromatograms of *F. verticillioides* had less than 40. Thousands of markers (accurate masses that may include fragments and abducts) were detected across samples making data interpretation extremely difficult. Compound identification was assessed through the creation of a local database and by searches in public domain databases. Principal Component Analysis (PCA), PLS-DA (Projection to Latent Structures-Discriminant Analysis) and semi-quantitative analysis of identified compounds were also undertaken demonstrating that integration of diverse strategies is mandatory to comprehensively understand complex metabolic data.

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Natural phenolic compounds as anti-aflatoxigenic and anti-fungal chemosensitizing agents

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Certain phenolic compounds, as antioxidants, prevent mycotoxin biosynthesis. For example, caffeic acid (12 mM) in a fatty acid-based growth medium prevents aflatoxin production by *Aspergillus flavus* NRRL 3357 by >95%, without affecting fungal growth. According to microarray analyses, expression of genes in the aflatoxin biosynthetic cluster is lowered in the caffeic acid-treated fungi from a $\log_2$ ratio of –0.04 (low) to –3.13 (high), compared to untreated fungi. Aflatoxin pathway regulator genes, *aflJ* or *laeA*, and genes within the sugar utilization cluster of treated fungi show only minor changes ($\log_2$ ratio 0.08 to –0.58). Expression of genes in amino acid biosynthesis, metabolism of aromatic compounds, *etc*., increases ($\log_2$ ratio > 1.5). The most striking increased expression ($\log_2$ ratio of 1.08 to 2.65) of genes occurs in four genes orthologous to *AHP1*, alkyl hydroperoxide reductase 1, a thioredoxin peroxidase of the peroxiredoxin family in *Saccharomyces cerevisiae*. Alternatively, organic peroxides, *tert*-butyl and cumene hydroperoxides, and hydrogen peroxide induce aflatoxin production. Induction of peroxiredoxins by antioxidants attenuates aflatoxigenesis in *A. flavus* through some yet to be defined signal-transduction pathway.

Alternatively, other phenolic compounds can serve as chemosensitizing agents to augment efficacy of fungicides or anti-fungal drugs by targeting genes in MAPK oxidative/osmotic stress response pathways. This chemosensitization results in significant augmentation of antifungal activity of certain fungicides and antifungal drugs. Such chemosensitization can also result in loss of resistance to antifungal agents in resistant strains of medically and agriculturally important fungi.

Pathways involved in resistance to the antifungal protein PAF of *Penicillium chrysogenum*

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The small, cationic and cysteine-rich protein PAF is secreted by *Penicillium chrysogenum* and exhibits growth inhibitory activity against numerous filamentous ascomycetes, including human and plant pathogens. We are interested in the elucidation of the mechanism of action of this antifungal protein. Our studies indicate, that at least two signaling cascades are implicated in the antifungal activity of PAF in the sensitive model organism *Aspergillus nidulans*. First, PAF activates the cAMP/protein kinase A cascade. A pkaA deletion mutant showed increased resistance to PAF and a pharmacological approach showed that PAF aggravated the effect of activators of the Pka signaling cascade and partially relieved the repressive effect of caffeine on this pathway. We could further show, that the protein kinase C/mitogen-activated protein kinase signaling cascade mediates basal resistance to PAF.

Additionally, we found that PAF severely disturbs calcium signaling. Minimal concentrations of calcium neutralized PAF toxicity in sensitive fungi. In *Neurospora crassa* expressing the calcium sensitive photoprotein aequorin a PAF induced elevation of the intracellular calcium resting level could be detected. The addition of the extracellular calcium-selective chelator BAPTA abrogated the PAF induced elevation of intracellular calcium, indicating that an influx of extracellular calcium is responsible for this effect. Notably, PAF mimicked the function of distinct calcium channel blockers.

Further examinations are in progress to unravel the question how these pathways are interconnected.

Farnesol induces the transcriptional accumulation of the *Aspergillus nidulans* Apoptosis-Inducing Factor (AIF)-like mitochondrial oxidoreductase

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Farnesol (FOH) is a non-sterol isoprenoid produced by dephosphorylation of farnesyl pyrophosphate, a catabolite of the cholesterol biosynthetic pathway. These isoprenoids inhibit proliferation and induce apoptosis. It has been shown previously that FOH triggers morphological features characteristic of apoptosis in the filamentous fungus Aspergillus nidulans. Here, we investigate which pathways are influenced through FOH by examining the transcriptional profile of A. nidulans exposed to this isoprenoid. We observed decreased mRNA abundance of several genes involved in RNA processing and modification, transcription, translation, ribosomal structure and biogenesis, amino acid transport and metabolism, and ergosterol biosynthesis. We also observed increased mRNA expression of genes encoding a number of mitochondrial proteins and characterized in detail one of them, the aifA, encoding the Apoptosis-Inducing Factor (AIF)-like mitochondrial oxidoreductase. The Delta-aifA mutant is more sensitive to FOH (about 8.0% and 0% survival when exposed to 10 and 100 microM FOH respectively) than the wild type (about 97% and 3% survival when exposed to 10 and 100 microM FOH respectively). These results suggest that AifA is possibly important for decreasing the effects of FOH and reactive oxygen species. Furthermore, we showed an involvement of autophagy and protein kinase C in A. nidulans FOH-induced apoptosis.

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Evolutionary genetics and genomics: Jason Stajich and Linda Kohn

Comparative Genomic Analysis of Pathogenic *Candida* Species

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We sequenced the genomes of six species related to the pathogenic yeast *Candida albicans* and used comparative analysis to identify features associated with pathogenicity and mating. Phylogenomic analysis across the related *Candida* and *Saccharomyces* clades finds significant expansions of cell wall, secreted, and transporter gene families in the pathogenic species, suggesting adaptations associated with virulence. Examination of polymorphisms within the diploid genomes reveals large homozygous tracts in *C. albicans*, *C. tropicalis*, and *Lodderomyces elongisporus*, possibly resulting from recombination during recent parasexual reproduction. Surprisingly however, key components of the mating and meiosis pathways are missing from several *Candida* species, particularly the haploid sexual ones, raising questions as to how mating and cell types are controlled in *Candida* species. Analysis of the evolutionary dynamics of CUG leucine to serine recoding reveals essentially all (99%) of the ancestral CUG codons were erased and new ones arose elsewhere. Lastly, conservation signatures were applied to revise the *C. albicans* gene catalog, identifying many new genes, most of which are *Candida* specific.

Insights into the evolution of fungal pathogenicity using comparative genomics

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Molecular phylogeny has shown that pathogenic fungi are found in many taxonomic groups, which suggests that these lifestyles have evolved repeatedly within the fungal kingdom. The availability of increasing numbers of fungal genome sequences has enabled comparisons to be made between the gene inventories of pathogenic and closely related saprophytic species of fungi in order to study the nature of pathogenesis. This has been aided by resources such as e-Fungi, a database that incorporates sequence and functional information from a number of fungal species, thus facilitating comparative genomic analyses (Cornell *et al*., 2007, Genome Res. 17: 1809-1822). By comparing the genomes of pathogenic and saprophytic species of filamentous ascomycetes, a number of gene families have been identified that are more highly represented in the proteomes / secretomes of phytopathogens, including putative effector proteins that might perturb host cell biology during plant infection. (Soanes *et al*., 2008, PLoS ONE 3: e2300). Using an evolutionary framework to compare the genomes of fungal species means that the contribution of gene gains, losses and duplications, as well as horizontal gene transfer to fungal evolution can be ascertained (Soanes *et al*., 2007, Plant Cell 19: 3318-3326). In addition, comparative genomics will help illuminate many areas of fungal evolution, such as the nature of obligate biotrophy and the role of innate immunity.

Multiple horizontal gene transfer events and domain fusions have created novel regulatory and metabolic networks in the oomycete genome.

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Complex enzymes with multiple catalytic activities are hypothesized to have evolved from more primitive precursors. Analysis of the *Phytophthora sojae* genome revealed 273 novel multifunctional proteins that are largely conserved across *Phytophthora* and *Pythium* genomes. Each of these proteins contains combinations of protein motifs that are not present in bacterial, plant, animal, or fungal genomes. Only 11% of these proteins models are also found in the diatom genome and thus the majority of these proteins have formed after the split between diatoms and oomycetes. We postulated that the novel multifunctional proteins of oomycetes might have value as potential Rosetta Stones to identify interacting proteins of conserved metabolic and regulatory networks in eukaryotic genomes. However ortholog analysis of each domain within the multifunctional proteins using the reciprocal smallest distance algorithm against 39 sequenced bacterial and eukaryotic genomes identified only 25 candidate Rosetta Stone proteins. Since the majority of proteins are not Rosetta Stones, they may instead serve to identify novel metabolic and regulatory networks in oomycetes. Since multifunctional proteins in metabolic pathways must act cooperatively with other components of the pathway, we looked in detail at the phylogenetic origins of enzymes in the sulfate assimilation, lysine and serine biosynthetic pathways. Each of these pathways had one or more bifunctional enzymes. Phylogenetic analysis of the proteins in these pathways revealed multiple examples of horizontal transfer from both bacterial genomes and the photosynthetic endosymbiont in the ancestral genome of Stramenopila. (Poster # 387)

Evolution of nonribosomal peptide synthetases: generating chemical diversity

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Non-ribosomal peptide synthetases (NRPSs) are multimodular enzymes that make non- ribosomal peptides (NRPs) through a thiotemplate mechanism independent of ribosomes. NRPs have important roles in both development and niche-specific success of filamentous fungi in addition to the well-known, useful, biological effects on other organisms including antibiotic, immunosuppressant, antitumor, and virulence-promoting activities. The modular structure of NRPSs, consisting of repeated units of Adenylation (A), Thiolation (T), and Condensation (C) domains, allows for both rapid evolution of novel genes, as well as flexibility in biosynthetic strategies. The mechanisms by which these genes evolve are likely complex, involving tandem duplication, duplication and loss, recombination, gene conversion, and fusion/fission of modular units (either single domains or A-T-C modules). We have addressed this issue, using several phylogenetic approaches in two datasets: 1) among homologs of the relatively conserved NRPSs that biosynthesize intracellular siderophores found in all filamentous ascomycetes and some basidiomycetes and 2) among NRPSs found in closely related Dothideomycete species. Our results suggest that 1) tandem duplication of complete A-T-C units represents the most plausible explanation for the generation of multimodular genes, 2) loss and/or swapping of A domains involved in substrate recognition may represent a mechanism for rapid evolution of new compounds, and 3) genes conserved across filamentous ascomycetes are also conserved in closely related species, while others appear prone to rapid duplication and rearrangement. (Poster # 66)

Mesosynteny between fungal chromosomes; a newly recognised type of sequence conservation found between fungal species.

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As new fungal genomes are sequenced and assembled, it becomes feasible to compare features between species. We have compared the Dothideomycetes, seeking patterns of genome conservation. We compared the nucleotide and predicted peptide sequence content of all scaffolds of the Dothideomycete wheat pathogen *Stagonospora nodorum* and other available assemblies. To our surprise, we observed a highly non-random pattern of sequence conservation. There was a strong tendency for coding regions in one scaffold to be grouped in one or a few scaffolds of other species. The order and orientation of the genes was heavily scrambled. This pattern, which was striking within the Pleosporales, was also noticeable in other Pezizomycetes but not yeasts. We call this pattern mesosynteny to distinguish it from macrosynteny such as observed between cereal chromosomes and microsynteny, which refers to the conserved gene-by-gene order and orientation. We observed mesosynteny with both heterothallic and homothallic species, but much less so with asexual species. The conservation of mesosynteny implies an evolutionary advantage to retaining sets of genes on one chromosome over evolutionary time. It allows the gene content of ancestral chromosomes to be determined. It can be used as a genome level phylogenetic tool. It also has implications as a potential short-cut in genome sequencing and assembly. (Poster # 101)

The evolution of ribosomal protein gene regulation in yeasts

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Ribosomal proteins' (RP) expression levels are tightly regulated in response to varying environmental and physiological states in the budding yeast *Saccharomyces cerevisiae*. Under normal growth conditions, genes that encode for structural constituents of the ribosome are among the most highly expressed in the cell. However, under environmental duress, these genes are repressed by a variety of factors. Here we use an integrated experimental and computational approach to study the impact of an ancestral whole genome duplication (WGD) among the *Ascomycota* lineage on the evolution of RP gene regulation under environmental stresses. These genes exhibit a prominent signature of paralogous gene retention and loss following the WGD. We focus on how the duplication and subsequent divergence of a key pair of paralogous transcriptional co-regulators, IFH1 (a co-activator) and CRF1 (a corepressor), may have impacted the transcriptional programs of their target genes. Significantly, these regulators share the same pattern of post-WGD gene retention as their targets, suggesting an intricate role of gene regulatory programs in modifying post-duplication genomic architectures. By probing the transcriptional dynamics of this module across five species from both pre- and post-WGD clades under numerous environmental stress conditions, we shed light on the regulatory evolution for an essential component of the cellular machinery. This analysis examines how diverse mechanisms can generate similar phenotypic responses throughout evolution.

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Genome-wide investigation of reproductive isolation in Neurospora: Candidate regions identified by microarray-based genotyping and mapping.

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Reproductive isolation is commonly caused by incompatibilities between genes from different species. Identifying the interacting genes, and determining their molecular functions and evolutionary histories, provides insight to the underlying components of the isolating mechanisms and the process of speciation. Experiments designed to instigate ecological speciation with Neurospora revealed the existence of a pair of incompatibility loci (dfe and dma) that cause perithecial abortion. This phenotype was severe and easily scorable, with alleles at both loci segregating in a Mendelian fashion, making these speciation genes a prime choice for further study. We investigated the dfe-dma incompatibility and how it relates to the genetics of reproduction isolation between N. crassa and N. intermedia. Controlled matings indicated which alleles of dfe and dma were possessed by each of these species. To map the approximate genomic location of the incompatibility loci, a new interspecific genetic map was constructed from ~1500 restriction-site associated DNA (RAD) markers, and three different approaches were taken. First, we applied the population genomics method of genome scanning to the experimental lineages. Second, we applied the quantitative genetics method of bulk segregant analysis to identity regions of the genome that consistently segregated with the appropriate incompatibility phenotypes. Third, we took the classical genetics approach of introgressing an N. intermedia allele into an N. crassa background and searching for genomic islands with divergent ancestry. By integrating the multiple lines of phenotypic, genetic, experimental, and molecular evidence, we demonstrate that the incompatibility loci map to two independent genomic regions, each representing ~2% of the nuclear genome.

Evolutionary dynamics and transcriptional landscape of *Neurospora crassa* revealed through RNA sequencing and comparative genomics

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Identifying the complete set of protein-coding and noncoding-RNA genes from an organism is critical for studying how the evolution of functional elements impact phenotypic evolution. The presence of small noncoding RNAs, an important aspect of developmental evolution in animals and plants, has not been thoroughly explored in *Neurospora*. The advent of low cost high throughput sequencing provides the means to sample these potentially hard to study transcripts. We updated the *N. crassa* gene annotations with transcript data from the Program Project and our RNA-Seq. This allowed us to make 3' and 5' UTR predictions for 60% of genes and highlighted additional transcriptionally active regions beyond the annotated protein coding genes. We sequenced 6M 20-40 bp tags of noncoding RNAs from wild-type *N. crassa* undergoing sexual development, growing vegetatively, and in liquid culture. We mapped these reads to the *N. crassa* genome and found an enrichment of loci overlapping 3'UTRs of ribosomal protein genes as well as several hundred locations with no currently identified genes. We also identified a subset of these loci that are located in highly conserved regions based on PhastCons scores of alignments of *N. tetrasperma* and *N. discreta* genomes. Some of these loci included known noncoding RNAs such as tRNAs and splicesomal RNAs. We have experimentally validated a handful of these loci to confirm their expression. Our work is a step towards a more complete description of the transcriptional landscape of *Neurospora*.

Dimorphic transitions and morphogenesis: Alex Andrianopoulos and Takashi Kamada

Regulation of *H. capsulatum* morphology and virulence in response to temperature

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We are studying how the fungal pathogen *Histoplasma capsulatum* changes its growth pattern to a virulent yeast form in response to the temperature of the host. We identified three genes, *RYP1*, *RYP2*, and *RYP3*, which are required for yeast-phase growth at 37ºC. Ryp1 is homologous to a family of fungal proteins including *Candida albicans* Wor1, which associates with DNA and regulates gene expression. Ryp2 and 3 are each homologous to the VeA family of proteins in filamentous fungi, which are also thought to regulate gene expression by unknown means. Each of the Ryp factors is differentially expressed at 37ºC, and each is required for expression of the other two. Notably, 98% of the yeast-specific gene expression program is dependent on Ryp1, indicating that Ryp1 is a key regulator of yeast-phase growth. We used chromatin immunoprecipitation-microarray (ChIP-chip) approaches to show that the Ryp factors associate with discrete genomic loci, presumably to regulate gene expression. Interestingly, the Ryp factors associate with the upstream regions of both morphology and virulence genes, suggesting that these factors are a molecular link that couples the expression of yeast-phase and virulence traits at 37ºC. Additionally, these data provide the first evidence that VeA homologs associate with DNA. We are currently annotating the entire set of Ryp-regulated genes, and intend to elucidate the upstream and downstream elements of the regulatory circuit that controls cellular behavior in response to temperature.

Control of white-opaque switching in *Candida albicans*

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The yeast *Candida albicans* is a harmless commensal in most healthy persons, but it can also cause superficial as well as life-threatening systemic infections, especially in immunosuppressed patients. *C. albicans* strains that are homozygous at the mating type locus can reversibly switch from the normal yeast form (white) to an elongated cell form (opaque), which is the mating competent form of the fungus. The two cell types also differ in their ability to colonize and infect various tissues; therefore, switching may allow *C. albicans* a better adaptation to different host niches. White-opaque switching occurs spontaneously at a low frequency and is controlled by several positively and negatively acting transcription factors, which form a transcriptional feedback loop that ensures the semi-stable maintenance of the two phases. However, white-opaque switching can also be induced by environmental signals, indicating that upstream regulators may control the activity of these transcription factors in response to such signals. A systematic search for regulators of white-opaque switching identified novel signaling pathways that control this important morphogenetic process in *C. albicans*.

The role of TOS9 domain proteins during growth and morphogenesis in the dimorphic human pathogen *Penicillium marneffei*

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Many fungal pathogens have the capacity to undergo dimorphic switching and this capacity is tightly linked to pathogenicity. Studies in *Candida albicans* identified the *wor1*/*tos9* gene as a master regulator of white/opaque (WO) switching (Zordan *et. al.*, 2006; Huang *et. al.*, 2006; Srikantha *et. al.*, 2006) and it has been show to function in a feedback loop with a number of other transcription factors known to be involved in the WO switch (Zordan *et. al.*, 2007). Mutagenesis studies in the dimorphic pathogen Histoplasma capsulatum identified the *ryp1* gene, a homologue of WOR1, and *ryp1* mutants are unable to switch from hyphal to yeast growth (Nguyen and Sil, 2008). Two further genes (*ryp2* and *ryp3*) were found to also be required for filamentous growth and are homologues of the Velvet A family of regulatory proteins in filamentous fungi (Webster and Sil, 2008).

The only known domain of RYP1 and WOR1 is the TOS9 domain. The founding member of this family is the *Schizosaccharomyces pombe pac2* gene which is involved in the regulation of mating (Kunitomo, *et. al.*, 1995).

Like *H. capsulatum*, the dimorphic human pathogen *Penicillium marneffei* also switches from hyphal to yeast growth with the switch of temperature to 37°C but divides by fission rather than by budding. *P. marneffei* has homologues of RYP1/WOR1 and of PAC2. The genes encoding these proteins have been cloned and characterised in *P. marneffei* using deletion and overexpression strains. In addition, their role in the regulation of target genes has also been examined in order to understand how they control growth and morphogenesis. (Poster # 227)

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Mechanisms of Flo11-dependent adhesion and morphogenesis in *S. cerevisiae*.

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The cell wall protein Flo11 governs adhesion and is an important determinant of cellular morphogenesis in *S. cerevisiae*. In various strains of yeast, Flo11 may be required for flocculation, invasion, pseudohyphae, biofilms, microbial mats, and unusual colony morphologies. We find, however, that only a subset of these phenotypes are displayed by each strain. In strain Sigma 1278b, for example, Flo11 is required for agar invasion but this strain does not flocculate. Strain *S. cerevisiae var. diastaticus*, on the other hand, requires *FLO11* for flocculation but does not invade agar. We are using these differences in strain-specific Flo11 phenotypes to investigate the mechanisms by which this adhesin determines morphology. We have completed the sequence of the *FLO11*gene from strains SK1 and diastaticus and compared them to the published sequences from strains S288c and Sigma 1278b. We find substantial differences in the sequences of the gene among these strains, particularly in the central domain which encodes tandem repeats. These repeats have been shown by us and others to be involved in adhesion. To test the importance of these variations on Flo11- dependent phenotypes we carried out gene swap experiments in which we replaced the *FLO11* gene of one strain with that of another. Most of these experiments revealed surprisingly modest effects of DNA sequence variation on phenotype, suggesting that other factors influence Flo11-dependent adhesion and morphogenesis. (Poster # 276)

A homeoprotein, NrsA represses sexual development of *Aspergillus nidulans*

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The *nrsA* gene is predicted to encode a novel 293 aa protein with a homeobox domain at the C terminus. Deletion of *nrsA* resulted in the increase of cleistothecial development even in the presence of stresses and in the reduction of conidia formation. Conversely, forced expression of *nrsA* blocked formation of cleistothecia completely. Over expression of *nrsA* in *nsdD* multicopy strain exhibited the phenotype similar to that of the wild type. And double over-expression of *nsdD* and *nrsA* inhibited both sexual and asexual development. These results suggest that NrsA act as a negative regulator of sexual development and may function as a member of potent antagonists of NsdD. The *nrsA* mRNA was hardly detectable during vegetative growth. It accumulated during asexual sporulation but was very unstable. More than ten GATA sequences were found in the promoter region of*nrsA*. *In vivo* ChIP assay revealed that the NsdD bound to the promoter of *nrsA*. The expression of *nrsA*increased in *nsdD* deletion mutant or *nsdD*/*veA* double deletion mutant, indicating that NsdD represses the transcription of the *nrsA* gene. Taken together, we propose that the homeo-domain protein, NrsA administrates coordinated regulation of two distinct developmental programs in *A. nidulans* and is under the antagonistic control of NsdD and VeA. (Poster # 186)

A molecular network of conserved signaling components and novel proteins controls cellular development in *Sordaria macrospora*

Ulrich Kück, Ines Engh, Sandra Bloemendal, Christian Schäfers, Jens Kamerewerd, Minou Nowrousian
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In filamentous fungi, fruiting body formation during sexual development is under polygenic control. We use the homothallic ascomycete *Sordaria macrospora* as a model organism to study cellular differentiation during vegetative and sexual development. Molecular genetic analysis of developmental mutants led to the identification of proteins controlling cellular differentiation. For example, these mutants lack novel not yet characterized PRO proteins (1,2) or have defective components of conserved signaling pathways (3). Using a genome-wide yeast two-hybrid screen, we identified novel interactions between PRO proteins and diverse cellular components. For example, PRO40, a protein previously demonstrated to associate with Woronin bodies interacts with the MAP kinase kinase MEK1 as well as the calcium binding protein PEF. These data indicate a link between conserved signaling components and developmental PRO proteins. Further molecular analysis will improve our understanding of the cellular network controlling sexual development in filamentous fungi. Our data suggest that PRO proteins act either as scaffolds, adaptors and/or activating proteins of conserved signaling pathways. (1) Engh I, Würtz C, Witzel-Schlömp K, Yu Zhang H, Hoff H, Nowrousian M, Rottensteiner H, Kück U (2007) Eukaryotic Cell 6: 831-843 (2) Nowrousian M, Frank S, Koers S, Strauch P, Weitner T, Ringelberg C, Dunlap JC, Loros JJ, Kück U (2007) Molec Microbiol 64: 923-937. (3) Kamerewerd J, Jansson M, Nowrousian M, Pöggeler S, Kück U (2008). Genetics 180: 191–206

Mutational analysis of sexual development in the mushroom *Coprinopsis cinerea*.

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In *Coprinopsis cinerea*, sexual development is under the control of the *B* and *A* mating-type genes. The *B* genes encode pheromones and their cognate receptors, controlling exchange and migration of nuclei between the mating partners for dikaryosis and induce the fusion of the clamp and subterminal cells to maintain the binucleate state within each cell in the dikaryon. The *A* genes encode two classes of homeodomain proteins, controlling conjugate division of the two nuclei associated with clamp connection in the dikaryon. The dikaryon grows to form a mycelial colony, which forms fruiting bodies under appropriate environmental conditions. Fruiting-body formation comprises (1) switching from mycelial growth to reproductive development, (2) cell differentiation into tissues and organs to form the fruiting-body primordium, (3) photomorphogenesis of the primordium, and (4) development of the primordium into the mature fruiting body, which consists of (i) meiosis & sporulation, (ii) elongation & gravitropism of the stipe, and (iii) expansion & autolysis of the cap. To identify the genes that work downstream of the mating-type genes and regulate dikaryon formation and/or fruiting-body morphogenesis, we have been isolating and analyzing mutations that affect the development. The genes we have identified so far include *clp1*, *clp2, pcc1* and *prd1*, which are involved in dikaryon formation, *frt1* involved initiation of fruiting, *ich1* involved in differentiation of the cap in the primordium, *dst1* and *dst2* involved in photomorphogenesis of the primordium, *eln1*, *eln3* and *eln6* involved in stipe elongation, *grv1* involved in gravitropism of the stipe, and *exp1* involved in cap expansion and autolysis.

Towards a better understanding of fruiting body development in basidiomycetous mushrooms

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A major goal of basidiomycetes research is to understand the molecular basis of fruiting body development. We have been using a battery of molecular techniques in both *Lentinula edodes* and *Coprinopsis cinerea* to study this important process. In *L. edodes*, we used RNA arbitrarily primed-PCR, SAGE, LongSAGE, EST sequencing coupled to dot-blot hybridization and cDNA microarray to analyze differentially expressed genes (DEGs) from various developmental stages. In *L. edodes*, in the transition from the mycelium to the primordium, (1) different hydrophobins were expressed abundantly, (2) fewer structural genes were expressed, (3) transcription and translation became active, (4) different genes became involved in intracellular trafficking, and (4) stress responses were expressed. In *C. cinerea*, with the aid of the genome sequence, we performed 5' SAGE to obtain and analyze 198,809 ditags from dikaryotic mycelium and primordium. The tags revealed the expression of 3,270 genes, 1,911 from mycelium and 2,732 from primordium. Among them, 1,040 were identified as DEGs. These DEGs showed that in *C. cinerea*, in the transition from mycelium to primordium, (1) genes in oxidative phosphorylation, nucleotide metabolism, TCA cycle, sugar metabolism, amino acids metabolism and protein transport expressed at higher levels, (2) the cAMP pathway was suppressed, (3) different adhesins were expressed higher, and (4) transcription and translation became active. Our studies provided a holistic understanding of the molecular basis of fruiting body development of the basidiomycetous mushrooms.

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Biofuels and biomass disassembly: Jonathan Walton and Scott Baker

Development of a recombinant Trichoderma strain for improved hydrolysis of pretreated corn stover

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A majority of currently available commercial enzyme products for biomass hydrolysis are produced by the saprophytic mesophilic fungus *Trichoderma reesei*. *Trichoderma* produces two cellobiohydrolases (CBHI and CBHII), five endoglucanases (EGs), and two beta-glucosidases (BGs). Although this mix is relatively efficient at cellulose degradation and large quantities of these proteins are secreted from the fungus, improvements in the total enzyme specific activity and secretion yield may improve this product. A primary factor in the high cost of enzymes for biomass hydrolysis is the amount of enzyme that must be applied for efficient cellulose conversion to glucose. Compared with starch hydrolysis, 15-100 fold more enzyme is required to produce an equivalent amount of ethanol, depending on specific process conditions. It is well known that efficient cellulose hydrolysis requires a complex, interacting mix of cellulose degrading proteins. To significantly reduce the enzyme loading required, one may replace *Trichoderma* components with more efficient candidates, or augment the enzyme system with additional components to improve the overall enzyme performance. In this study, identification of new genes that improve specific performance in hydrolysis of pretreated corn stover, and their expression in *Trichoderma* will be discussed. (Poster 626)

Mechanisms of lignocellulose degradation inferred from transcriptome and secretome analysis of the wood decay fungi *Phanerochaete chrysosporium* and *Postia placenta*.

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The wood decay basidiomycetes *Phanerochaete chrysosporium* and *Postia placenta* were grown under various culture conditions and subjected to whole genome expression microarray analysis and liquid chromatography tandem mass spectrometry (LC-MS/MS). Hundreds of genes were flagged on the basis of significant changes in transcript accumulation and/or extracellular peptide sequence. In medium containing microcrystalline cellulose as sole carbon source, 28 *P. chrysosporium* genes encoding carbohydrate-active enzymes (CAZYs) were significantly upregulated relative to glucose-containing medium. On the basis of structure and MS-derived peptide sequence, 10 of these genes were confidently identified as exo- or endo- cellulases. Consistent with an unconventional mechanism of cellulose depolymerization, only 1 of the 18 upregulated CAZY-encoding genes of *P. placenta* was categorized as a potential cellulase. Presenting a daunting challenge for future research, 24 *P. chrysosporium* genes and 154 *P. placenta* genes are upregulated under cellulolytic conditions but predicted to encode proteins with no significant similarity to known proteins. Many of these hypothetical proteins feature secretion signals, and in 12 cases, the corresponding protein has been identified in culture filtrates by MS. Likely, some of these genes play an important role in lignocellulose degradation.

Genome mining to improve bio-ethanol pre-treatments

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The first step in bio-ethanol production involves hydrolysis of plant polysaccharides to fermentable sugars using either chemical or enzymatic treatment. The currently available enzyme cocktails for bio-ethanol pre-treatment leave a significant part of the biomass unused. The availability of a growing number of fungal genomes provides a large variety of potential novel enzymes that may improve these enzyme cocktails. Detailed analysis of fungal genomes enables reliable function prediction for many putative plant polysaccharide degrading enzymes as was evidenced recently for *Aspergillus nidulans*, where the function prediction was confirmed by large scale enzyme analysis. Fungal comparisons highlight enriched enzyme families that can be related to the natural biotope and the ability of the species to grow on different polysaccharides. Identification of genes encoding enzymes for which no previous genes have been identified is a more complex issue. In addition, characterization of the in vivo function of novel enzymes relies on specific substrates that can distinguish between small differences in activity between iso-enzymes. Finally, production of the enzymes depends on improved (fungal) production systems that can not only produce enzymes of ascomycete origin, but also those derived from basidiomycetes and zygomycetes.
Recent strategies and examples will be discussed to highlight the possibilities and limitations of genome mining for the identification of novel enzymes that could be used to improve bio-ethanol pre-treatments.

Approaches to understanding fungal cellulase action in biomass disassembly

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Lignocellulosic biomass is the most plentiful, least expensive renewable natural resource for producing biobased products and bioenergy. The structure and chemical composition of plant cell walls have evolved to be resistant to enzymatic conversion. As a result, an effective pretreatment is needed that enables the efficient cellulose conversion by hydrolytic enzymes. Fungi may be the most effective decomposers of plant biomass and have evolved to have highly diverse hydrolytic enzymes enabling them to use native biomass substrates. Pretreatment can change characteristics such as crystallinity, morphology, and substrate porosity. These changes directly impact the types of enzymes needed for efficient hydrolysis and impacts how cellulase mixtures are improved through protein engineering based on either rational design or directed evolution.

***Trichoderma reesei* as a production organism for enzymes for biomass hydrolysis.**
Merja Penttilä, VTT Biotechnology, Espoo, Finland

During decades *Trichoderma reesei* has been used as a model fungus in enzymology for cellulose and hemicellulose hydrolysis and an important industrial host for enzyme production. It is currently one of the most important organisms for production of enzymes mixtures for efficient total hydrolysis of pant biomass to fermentable sugars needed in biorefiny applications such as bioethanol production. We have analysed optimal enzymes for sugar generation of various different feedstocks such as corn stover and steam exploded spruce. Protein engineering has been used to generate more stable and active enzymes. We have also analysed the gene expression profiles of *Trichoderma* in cultures containing various technical biomass substrates, which shows significant regulation capacity by the fungus to induce specific enzyme sets against different plant raw material preparates.

Strain improvement of Trichoderma using green fluorescent protein and fluorescence activated cell sorting
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The filamentous fungus *Trichoderma reesei* is an important source of hydrolytic enzymes in nature and industry. Germlings of *Trichoderma reesei* were used for directed evolution to improve secretion of enzymes for industrial production using high speed fluorescence activated cell sorting (FACS). To determine if flow cytometric measurements of protein expression could be made on germlings we created a gene construct placing the *Renilla reniformis* green fluorescent protein (GFP) gene under control of the cellobiohydrolase I (cbh1) promoter and terminator of *T. reesei*. This vector was transformed into the genome of *T. reesei* and GFP expression was measured in germlings by flow cytometry. Green fluorescence was observed in germlings grown under conditions known to produce cellulase expression in *Trichoderma*. Spores were mutated with UV light and growing germlings were screened using FACS, yielding improved mutants. (Poster # 293)

Post translational modification of AoXlnR, a key transcriptional regulator of biomass- degrading enzymes in *Aspergillus oryzae*.
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We previously identified *A. oryzae* XlnR (AoXlnR) as a transcriptional regulator of cellulase and xylanase genes. Anlalysis using *A. oryzae* oligonucleotide array revealed that it activated transcription of 38 enzyme genes possibly involved in cellulose and xylan degradation and D-xylose metabolism upon induction by D-xylose. Since cellulose and xylan are two major plant polysaccharides, AoXlnR appears to be a most important regulator of biomass degrading enzyme genes. The purpose of our current research is to clarify the molecular mechanism of AoXlnR- mediated transcriptional regulation. We found that AoXlnR was reversibly modified in the presence of D-xylose. Calf Intestine Alkaline Phosphatase treatment revealed that it was phosphorylated both in the presence and absence of D-xylose, and that the degree of phosphorylation was higher in its presence. Furthermore, AoXlnR in the presence of D- xylose had a higher molecular weight than that in its absence even after the phosphatase treatment. These results indicated that AoXlnR is constitutively phosphorylated and that D-xylose triggers additional phosphorylation and also an unknown modification. We are now trying to identify the phospholyration sites and the unknown modifier. (Poster # 113)

Sex and the *Trichoderma*: New perspectives for industrial strain improvement.
Verena Seidl, Christian Seibel, Christian P. Kubicek and Monika Schmoll. Institute of Chemical Engineering, Vienna University of Technology, Austria. Email: vseidl@mail.tuwien.ac.at.

The species *Trichoderma reesei* is industrially used for the production of cellulolytic and hemicellulolytic enzymes. All mutant strains used for biotechnological applications and research have been derived from one asexually propagating isolate, QM6a. The possibility to carry out sexual crossings with *T. reesei* QM6a would be highly desirable for basic research and industrial strain improvement. *Hypocrea jecorina*, a heterothallic species, was described to be the sexual form of *T. reesei*, but previous attempts of sexual crossings between *T. reesei* QM6a and *H. jecorina* strains were not successful. The aim of this study was to (re-)address the question if the industrial workhorse *T. reesei* QM6a is really an asexual clonal line. Analysis of the genome database revealed a *MAT1-2* mating type locus. We were able to isolate strains of the opposite *MAT1-1* mating type from a *H. jecorina* wild-type isolate. Upon mating with *T. reesei* QM6a those strains produced stromata and mature ascospores, thus enabling us for the first time to obtain sexual crossings with industrially used *T. reesei* strains. Both *MAT* loci from *H. jecorina* were characterized and we were able to convert the *MAT1-2* into the *MAT1-1* mating type in *H. jecorina* by targeted gene replacement and also successfully introduced the opposite mating type locus into *T. reesei*. (Poster # 413)

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CONCURRENT SESSIONS III

Fungal and oomycete effectors: Paul Birch and Thierry Rouxel

Effectors of a xylem colonizing fungus

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The pathogenic fungus *Fusarium oxysporum* f.sp. *lycopersici* (Fol) secretes enzymes and small proteins into xylem sap during colonization of its host, tomato. Several of these proteins have been identified using mass spectrometry. Among the eleven small secreted proteins identified are three that can trigger effector-mediated immunity: Avr1, Avr2 and Avr3 that are recognized by the resistance proteins I, I-2 and I-3, respectively. Furthermore, Avr2 and Avr3 contribute to virulence towards susceptible plants. Avr1 does not, but instead suppresses I-2 and I-3-mediated resistance. Single point mutations in Avr2 prevent recognition by I-2 but do not affect its virulence function. Part of the arms race between Fol and tomato can now be tentatively reconstructed.
Avr2 can be recognized intracellularly by I-2 (an NBS-LRR protein) leading to cell death in leaves of *Nicotiana benthamiana* (upon agroinfiltration and co-expression with *I-2*) and in *I-2* containing tomato (using a PVX-based expression system). We hypothesize that Avr2, and possibly other effectors, are taken up by xylem contact cells and subsequently either suppress basal resistance (susceptible host) or trigger R gene based defences.

Unraveling the mechanism of RxLR mediated translocation of Oomycete effector proteins.

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Most oomycete pathogens invade their hosts in a biotrophic manner, which means that they try to avoid host recognition and/or suppress host immune responses. Several biotrophic pathogens translocate effector-proteins into their host cells, which help to establish a successful infection. Oomycete pathogens do not possess a type III secretion machinery as bacteria have, and instead they seem to have developed a different effector translocation system. They are able to translocate proteins that contain an RxLR-EER motif located after the signal peptide. It was shown that this motif is important for effector translocation as mutating this domain stops translocation into the host cells. The mechanism by which oomycetes direct their RxLR-EER effectors into host cells is as yet unknown and is the main focus of our research. It has been postulated that endocytosis processes or protein transporters are responsible. Here we present our latest results, which give insight into the mechanism of the oomycete RxLR-EER protein translocation system.

The effectors of smut fungi: from comparative genomics to function.

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The basidiomycete fungus *Ustilago maydis* is a biotrophic maize pathogen that does not use aggressive virulence strategies and needs the living plant tissue for completion of its life cycle. The *U. maydis* genome codes for a large set of novel secreted effector proteins. Many of the respective genes are clustered in the genome and are upregulated during pathogenic development? About half of these gene clusters have crucial roles during discrete stages of biotrophic growth. We now show that *U. maydis* is eliciting distinct defense responses when individual clusters/genes are deleted. Maize gene expression profiling has allowed us to classify these defense responses and provides leads to where the fungal effectors might interfere. We describe where the crucial secreted effector molecules localize, their interaction partners and speculate how this may suppress the observed plant responses. In addition, we will present insights on effector evolution that stems from a comparative genomics approach in which the genomes of a second *U. maydis* strain as well as the genomes of the related smuts *Sporisorium reilianum* and *U. scitaminea* were sequenced using 454-technology.

Functional analysis of Phytophthora infestans RXLR effectors Avr2 and Avr3a

Eleanor Gilroy[1], Miles Armstrong[1], Jorunn Bos[2], Ros Tayor[1,3], Juan Morales[1], Ingo Hein[1], Emma Douglas[1], Petra Boevink[1], Anna Avrova[1], Susan Breen[1], Pieter van West[4], Leighton Pritchard[1], Stephen Whisson[1], Ari Sadanandom[3] , Sophien Kamoun[2] & Paul Birch[1,5]. [1]Plant Pathology Programme, SCRI, Dundee DD2 5DA,UK. [2]The Sainsbury Lab, JIC, Norwich, NR4 7UH, UK. [3]BLS, University of Glasgow, Glasgow G12 8QQ, UK. [4]Aberdeen Oomycete Group, University of Aberdeen, IMS, Aberdeen AB25 2ZD, UK. [5]Division of Plant Sciences, University of Dundee at SCRI, Dundee DD2 5DA, UK.

AVR3a, the first effector characterized from Phytophthora infestans, contains an N- terminal RxLR and dEER motif required for transport across the host plasma membrane. Genomic resources have revealed approximately 500 rapidly diverging RXLR effectors. Transient expression of effectors in collections of cultivated and wild Solanum species identifies sources of resistance (R) genes that recognise specific effectors. By silencing effectors in P. infestans we identified >15 functionally essential and >10 redundant for virulence. To examine effector functions we are identifying interacting plant host proteins. Subcellular localisations of effectors and targets are being uncovered using fluorescent labels. We will present our progress in the investigation of pathogenicity functions of the AVR2 and AVR3a avirulence effectors. We will present data visualizing translocation from haustoria and showing that these effectors interact with different host proteins to establish infection. Approaches such as virus-induced genes silencing, are being used to determine the roles of host targets in defence. (Poster # 510)

AT-rich isochores as ecological niches for effectors in the genome of *Leptosphaeria maculans*

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The genome of the ascomycete *Leptosphaeria maculans* shows the unusual characteristics to be organized in isochores, i.e., the alternating of homogeneous GC% regions with abrupt changes from one to the other. GC-equilibrated isochores (average 52% GC) are gene-rich whereas AT-rich isochores (40-43% GC) are mostly devoid of active sequences and are made up of mosaics of intermingled and degenerated repeated elements. The three avirulence (*AvrLm*) genes identified so far in this species are “lost in middle of nowhere” genes, isolated in the middle of large AT-rich isochores. Our postulate thus was that AT-rich isochores were specific “ecological niches” for avirulence genes and effectors in *L. maculans*. This was firstly validated by analysis of three genes lying in the same genome environment (*LmCys* genes) and showing the same characteristics as *AvrLm* genes (low GC content, strong overexpression at the onset of plant infection, encoding for small secreted proteins -SSP- often rich in cysteines). Of these, one, *LmCys2*, was shown to act as an effector, probably contributing to suppression of plant defense. On these bases a systematic search for SPP as effector candidates was performed using bioinformatics. 455 AT-rich isochores were extracted from the genome data and their repeat content masked using the *L. maculans* repeated element database. Non-repeated regions were then investigated with a pipe-line dedicated to the identification of SSP. This provided us with three datasets: 529 SSP-encoding genes in GC-equilibrated isochores, 498 non-SSP- and 122 SSP-encoding genes in AT-rich isochores. Part of this latter set of genes was analyzed for their occurrence in natural populations and expression data in culture and in planta. Finally, the 122 AT-SSP putative proteins showed structural features reminiscent of the *AvrLm* and *LmCys* genes. Possible diversification mechanisms as a function of genome location will be discussed.

Crinklers: A second class of host translocated effectors from oomycete plant pathogens.

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It is now well established that oomycete plant pathogens secrete effectors that are translocated inside host plant cells to enable parasitic infection. One class of these so-called cytoplasmic effectors, the RXLR effectors, carries a conserved motif that is located downstream of the signal peptide and has been implicated in host translocation. This presentation will report on the Crinkler (CRN) family, a second class of cytoplasmic effectors of oomycetes. The CRNs were originally identified from *Phytophthora infestans* transcripts encoding potentially secreted peptides that elicit host necrosis and alter immunity when expressed *in planta*. Analysis of the *P. infestans* genome sequence revealed an enormous family of 196 CRN genes of unexpected complexity and diversity. The CRNs are also the most expanded gene family in *P. infestans* relative to *Phytophthora sojae* (100 members) and *Phytophthora ramorum* (19). Similar to the RXLRs, CRNs are modular proteins. They are defined by a highly conserved N-terminal ~50 amino acid LFLAK domain and an adjacent, diversified DWL domain. Most (60%) CRNs possess a predicted signal peptide. The N-terminal regions of several CRNs are functionally interchangeable with the N-terminus of RXLR effectors and can mediate host translocation of AVR proteins. The CRN C-termini are remarkably diverse, exhibiting a wide variety of domain structures with strong evidence of recombination between different clades. We assayed representative genes from the *P. infestans* CRN families, and identified several distinct C-termini that trigger cell death inside plant cells. These include domains with similarity to protein kinases and phosphotransferases. Altogether, our results suggest that the CRN proteins are translocated inside plant cells and stimulate cell death via intracellular mechanisms.

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Secreted effectors of the tomato leaf mould fungus *Cladosporium fulvum* are virulence factors that target host defense

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Cladosporium fulvum is a biotrophic fungal pathogen that causes leaf mould of tomato. Inside the leaf, *C. fulvum* does not penetrate host cells or develop haustoria but remains confined to the intercellular space between mesophyll cells. Ten effector proteins that are secreted during host colonization have been identified so far. We have recently revealed virulence functions for a number of these effectors. It was previously shown that the Avr2 effector interacts with, and inhibits, the tomato cysteine protease Rcr3 which, in compliance with the guard hypothesis, is required for immunity mediated by the tomato resistance protein Cf-2. However, in compatible interactions Avr2 inhibits several additional extracellular host cysteine proteases that are required for host basal defense. Also the recently identified secreted effector Ecp6 is a true virulence factor, as RNAi- mediated gene silencing compromised fungal virulence on tomato. Intriguingly, all previously identified *C. fulvum* effectors are unique as no clear homologs have been identified in other organisms. Ecp6 is an exception to this rule, with clear homologs in many fungal species. Ecp6 contains LysM domains that have been implicated in carbohydrate binding and we speculate that Ecp6 plays a role to dampen host immune responses. A detailed characterization of the secreted effectors will be presented. (Poster # 527)

Recognition of *Phytophthora infestans* RXLR-dEER effectors by resistance proteins is triggered by C-terminal domains comprising W motifs

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The *Phytophthora infestans* avirulence genes *PiAvr1* and *PiAvr4* encode RXLR-dEER effector proteins and belong to a family of oomycete avirulence homologs (*Avh*). Avh proteins are rapidly evolving but nevertheless, the majority has recognizable C-terminal motifs (Jiang et al. 2008 PNAS). *PiAvr4* was isolated by positional cloning. Loss of avirulence on *R4* potato is caused by frame shift mutations resulting in truncated PiAvr4 proteins (van Poppel et al. 2008 MPMI). The genomic region harboring *PiAvr4* shows conserved synteny with *Phytophthora sojae* and *P. ramorum* but *PiAvr4* itself is located on a 100 kb indel that breaks the conserved synteny, and is surrounded by transposons. In the C-terminus PiAvr4 has three W motifs and one Y motif. W2 in combination with either W1 or W3 triggers necrosis in potato plants carrying resistance gene *R4*. *PiAvr1* was isolated by anchoring *Avr1*-associated markers on the genome sequence. This lead to a 800 kb region with seven *Avh* genes, one of which is *PiAvr1*, the counterpart of resistance gene *R1*. Also PiAvr1 has W and Y motifs. Domain swapping revealed which motifs determine avirulence on *R1* potato. Analysis of the role of PiAvr1 and PiAvr4 in virulence is in progress. (Poster # 530)

Phylogenomics: Joey Spatafora and David Geiser

Phylogenetic informativeness and the fungal tree of life.

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During the project "Assembling the Fungal Tree of Life" (AFTOL) the focus was to maximize the number of taxa representing the fungal kingdom and sequence genes that were generally used in phylogenetic studies (protein coding: RPB1, RPB2, TEF1, non-protein coding: SSU, LSU mSSU). Analysis of these concatenated gene alignments resulted in the discovery of new clades and relationships. While a number of longstanding questions were answered, many of the deep nodes in the phylogeny still lack resolution. Now, numerous sequenced genomes representing diverse fungal clades provide the opportunity to select genes which will inform us about these ancient divergences. Consequently, to add resolution to the evolutionary history of the Fungi (AFTOL2) the current project will focus on a few representative taxa (~200) and maximize the number of genes (~25). Using our own pipeline of scripts (Hal), universal, low copy homologous genes were identified and alignments constructed in an automated process. For the sake of comparison we also selected and aligned genes used in other studies and ATOL projects. The phylogenetic informativeness of each gene was assessed. This method involves quantification of informativeness of characters across historical epochs. This quantification enabled us to rank genes with characters that evolved close to the optimal rate distribution for the time period of our interest. Hal selected genes were more cost-effective in terms of phylogenetic informativeness than those protein coding genes used in the previous AFTOL project. The majority of Hal selected genes were more informative per site compared to amino acid alignments of protein coding genes used in other studies and ATOL projects.

Functional differentiation of fungal chitinases.

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Chitin is the second most abundant natural polysaccharide found in nature and it is a major component of the fungal cell wall. Chitinases are enzymes that are capable of degrading chitin directly to low molecular weight products by hydrolyzing the glycosidic bonds. Chitinases also have aggressive roles as fungal pathogenicity factors during infection of other fungi. Based on their amino acid sequence, chitinases are divided into two different glycoside hydrolase families (18 and 19) which are further subdivided into groups. Fungal chitinases belongs to glycoside hydrolase family 18. Analyses of complete fungal genome sequences have revealed a large diversity of novel types of putative chitinases. Subgroup CI and CII chitinases contain LysM peptidoglycan binding domains and chitin-binding type 1 domains. They show similarity with the yeast killer toxin from *Kluyveromyces lactis*, and are therefore believed to be involved in antagonistic interactions with other fungi. Knockout mutants of CII chitinases are constructed by homologous gene targeting in *Aspergillus nidulans*. In addition, knockout mutants of CII chitinases in *Neurospora crassa* are screened for different phenotypic characters. In another study, Green Fluorescence Protein is going to be used as a tag for CII chitinases in *A. nidulans* to study spatial and temporal expression patterns during antagonistic interactions. (Poster #233)

Carbohydrate-active enzymes in fungal genomes.

Bernard Henrissat, CNRS, Universités Aix-Marseille

Due to the extreme variety of monosaccharide structures, to the variety intersugar linkages and to the fact that virtually all types of molecules can be glycosylated (from sugars themselves, to proteins, lipids, nucleic acids, antibiotics, etc.), the large variety of enzymes acting on these glycoconjugates, oligo- and polysaccharides probably constitute one of the most structurally diverse set of substrates on Earth. Collectively designated as Carbohydrate-Active enZymes (CAZymes), these enzymes build and breakdown complex carbohydrates and glycoconjugates for a large body of biological roles. In fungi, these enzymes control cell wall synthesis and remodelling, plant polysaccharide digestion, detoxification of phytoalexins, glycogen and trehalose metabolism, and various house-keeping functions such as protein glycosylation. We have created and maintain the carbohydrate-active enzymes database (www.cazy.org), a resource dedicated to these enzymes, since 1998. Here we will briefly describe how the carbohydrate-active enzyme spectrum encoded by fungal genomes correlates with symbiont, parasite, pathogen or saprophyte lifestyles.

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Excavating the adaptive palimpsest: acquisition and evolution of ecological function in fungal genomes.

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Fungi, like prokaryotes, can obtain peripheral metabolic pathways through horizontal transfer of gene clusters. Subsequent genomic evolution may be characterized by rearrangement and loss of the genes that make up these clusters and functional diversification of the constituent gene families. We examined peripheral metabolic gene cluster evolution in basidiomycete and ascomycete lineages and evaluated evidence of functional diversification and positive selection that may reflect fungal niche adaptation at the interface between genome and environment. (Poster # 37)

SNPs of information: Inferring evolutionary history in *Coccidioides*.

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Coccidioides spp., the causative agent of Coccidioidomycosis, is a dimorphic fungus, with a saprobic hyphal phase and a pathogenic spherule phase. We have previously shown that there are two species of *Coccidioides*, *C. immitis* (found in California and Mexico) and *C. posadasii* (found in Arizona, Texas, Mexico and South America). Recently, 4 strains of *C. immitis* and 10 strains of *C. posadasii* have been sequenced. Using a set of high-quality SNPs in these genomes, we have investigated effective population size, comparative SNP rates across different genomic features, patterns of positive selection in coding regions, and levels of conservation among vaccine candidates. We see that *C. posadasii* has a 2-fold larger effective population compared to *C. immitis*, but that *C. immitis* has more genes undergoing positive selection. Additionally, we have identified a set of conserved potential vaccine candidates. Using these data, we can make further hypotheses about the evolutionary history of *Coccidioides spp.* and inform the development of vaccine candidates. (Poster # 429)

Assessing the performance of single-copy genes for recovering robust phylogenies

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Phylogenies involving nonmodel species are based on a few genes. Because gene trees are sometimes incongruent with species trees, the resulting phylogenies may not accurately reflect the evolutionary relationships among species. The increase in availability of genome sequences now provides large numbers of genes that could be used for building phylogenies. However, only a few genes can be sequenced for a wide range of species. Here we asked whether we can identify a few genes, among the single- copy genes common to most fungal genomes, that are sufficient for recovering accurate and well-supported phylogenies. Using 21 complete, publicly available fungal genomes with reliable protein predictions, 246 single-copy orthologous gene clusters were identified. We inferred the maximum likelihood trees using the individual orthologous sequences and constructed a reference tree from concatenated protein alignments. The topologies of the individual gene trees were compared to that of the reference tree using three different methods. The performance of individual genes in recovering the reference tree was highly variable. Two genes recovered exactly the same topology as the reference tree, and when concatenated provided high bootstrap values. The genes typically used for fungal phylogenies did not perform well, which suggests that current fungal phylogenies based on these genes may not accurately reflect the evolutionary relationships among species. Aguileta et al. Syst. Biol. 57(4):613–627, 2008 (Poster #401)

Regulatory subfunctionalization and neofunctionalization account for the preservation of the ancient and extended cutinase family in *Magnaporthe oryzae*.

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The cuticle is the first barrier for fungi that parasitize plants systematically or opportunistically [1]. The functional characterization of three members of the cutinase family [2,3] of the destructive rice blast fungus, *Magnaporthe oryzae* [4] and the over-representation of cutinases in the sequenced genomes of Pezizomycotina render this a suitable multigene family to study the evolution of functional divergence of duplicates in fungal pathogens [5]. The family of cutinases shows extreme sequence diversity and is divided in two ancient subfamilies which predate the split between the two major fungal phyla, Ascomycota and Basidiomycota [1]. We discuss factors affecting the gene family size of the cutinase families between five Ascomycetes: the phytopathogens *M. oryzae*, *Fusarium graminearum* and *Botrytis cinerea*; and the model *Neurospora crassa* and *Aspergillus nidulans*. The average ratio of cutinase gene gain to loss is 2:3, with the exception of *M. oryzae* and *N. crassa*, which exhibit extreme family expansion and contraction, respectively [1]. The regulatory subfunctionalization and neofunctionalization of most *M. oryzae* cutinase gene pairs provide the first justification for the retention of paralogs after duplication and for gene redundancy in the genomes of fungal pathogens [5].

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Comparative analysis of transposable elements in several fungal genomes

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Transposable Elements (TEs) are repeated sequences whose abundance is usually correlated with genome size. TEs shapes genomes and are a source of genetic variations and mutations. We started a comparative genomic analysis of fungal TEs using a rigorous annotation strategy. The challenge is to detect *ab initio* TEs and annotate them including nested and degenerated copies. We use the REPET pipeline developed at URGI which efficiently detects and annotates TEs in novel genomes. We will present preliminary results obtained with REPET on *Botrytis cinerea, Sclerotinia sclerotiorum, Leptosphaeria maculans, Tuber melanosporum* and *Blumeria graminis*. Searching for *B graminis* TEs using RepeatMasker against RepBase Update (known TEs) showed that they represent only 10% of this genome whereas REPET analysis showed up to 75%, TEs mainly as novel elements not already described in RepBase, illustrating the interest of such a pipeline. (Poster # 11)

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ROS in development and pathogenesis: Paul Tudzynski and Wilhelm Hansberg

ROS, RAS-1, growth and development.

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In *Neuropspora crassa*, conidiation is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: hyphae adhesion, aerial hyphae growth and conidia development. Each transition is started by an unstable hyperoxidant state and results in growth arrest, autophagy, an antioxidant response and a dioxygen insulation process. These responses stabilize the system and, once stable, growth can start again. We hypothesized that RAS-1 acts as a switch between growth and cell differentiation. The "band" mutant (*bd*) has a dominant *ras-1* mutation that results in the alternation of growth and conidiation. *ras-1*^{bd} has an inappropriate signaling through the MAK-2 and OS-2 kinases and develops increased oxidative stress during conidiation. RAS-1 altered signaling affects both growth and cell differentiation. (Poster #231)

Role and regulation of the Nox family in the filamentous fungus *Podospora anserina*

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For several years, we use the coprophilous fungus *Podospora anserina* to study the signalling pathways regulating the various stages of the fungal life cycle, i.e., mycelium growth and differentiation, fruiting body development and ascospore maturation and germination. We have identified NADPH oxidases/Nox, a family of enzymes dedicated to ROS production as key signaling players. Three genes coding such enzymes are present in the *P. anserina* genome. We have shown that these enzymes are involved at almost all steps of the life cycle, since PaNox1 is necessary for proper mycelium and fruiting body development and PaNox2 is necessary for ascospore germination, but also for efficient nutrient scavenging during the trophic phase. I will present the additional data that we have recently obtained regarding the cellular function of PaNox1 and PaNox2 and what we know about the third isoform, PaNox3. I will try to integrate the role of these enzymes in the broader context of the regulatory networks acting during fruiting body and ascospore development in *P. anserina*. This work is funded by contract # ANR-05-BLAN-0385-01 from the ANR

Determining the role of reactive oxygen species generation in *Magnaporthe grisea*

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NADPH oxidases (Nox) are flavoenzymes used to generate reactive oxygen species (ROS). Until recently, research into Nox and ROS generation was focussed primarily on the oxidative burst associated with the plant defence response and leukocyte function. However, the discovery of new functional members of the Nox family in filamentous fungi has implicated ROS in many diverse processes including cellular differentiation and sexual development. Here, we investigate the role of NADPH oxidase-generated ROS in the infection-related development of the phytopathogenic ascomycete fungus *Magnaporthe oryzae*. The formation of a specialised cell known as an appressorium allows the fungus to breach the host cuticle using mechanical force and subsequently cause plant infection. This project has focused on functional characterization of three NADPH oxidase homologues found within the *M. grisea* genome. We have demonstrated that Nox1 and Nox2 are independently required for pathogenicity of *M. grisea* and contribute to appressorium morphogenesis and function and appear to be regulated by Rac1 and a putative NoxR-encoding gene. Nox3 meanwhile plays a role in hyphal elongation and the regulation of conidiogenesis and potentially encodes a calcium-regulated form of Nox. When considered together these data suggest that Nox proteins regulate cellular morphogenesis and virulence-associated development in *M. oryzae.*

Bem1 and Cdc24: additional components of the *Epichloë festucae* NADPH oxidase complex?

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Epichloë festucae is a biotrophic fungus that forms a mutualistic symbiotic interaction with perennial ryegrass, *Lolium perenne*. Stable maintenance of this association requires functional copies of NoxA, NoxR and RacA (Tanaka et al. 2006, 2008; Takemoto et al. 2006). NoxR has two protein interaction domains, an N-terminal TPR domain for interaction with RacA and a C-terminal PB1 domain, suggesting that NoxR acts as a scaffold for the Nox enzyme complex. Given PB1 domains are known to interact with other PB1 domains, fungal genome databases were interrogated for proteins containing this domain to identify potential candidates that interact with NoxR. Proteins identified included NoxR itself, Cdc24, Bem1 and a protein of unknown function Yeast two-hybrid analysis showed that NoxR interacts with itself and Cdc24, and Cdc24 interacts with Bem1. Site directed mutagenesis of conserved residues in the K and OPC motifs of the PB1 domains of NoxR, Bem1 and Cdc24, confirmed the specificity of these interactions. An *E. festucae Dbem1* mutant was generated and shown to be defective in vacuolar fusion and the ability to form conidia. Perennial ryegrass plants infected with *Dbem1* had shorter leaves and more tillers than wild-type but unlike plants infected with *DnoxA*, did not senesce. Multiple hyphae were observed in many of the intercellular spaces compared to wild-type. GFP fusions of NoxR and Bem1 preferentially localized to the hyphal tip. The role of these proteins in controlling polarized growth will be discussed.

Role of reactive oxygen species and stress signaling pathways in development and virulence of *Cochliobolus heterostrophus*.

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Reactive oxygen species (ROS) induce cellular stress pathways, as well as provide developmental cues. Host and environmental signals modulate sexual and asexual sporulation, appressorium formation and virulence of the maize pathogen *Cochliobolus heterostrophus*. To study the signaling network linking stress and developmental responses, we identified genes encoding NADPH oxidases (NOX), superoxide dismutases (SOD), Rac GTPases and upstream members of the MAPK modules. Search of the *C. heterostrophus* strain C5 genome indicates 3 NOX, 3 Cu/Zn SOD and 3 Fe/Mn SOD genes. *noxA*, *noxB*, and *noxC* mutants grow normally; noxA mutants show reduced pigmentation and sporulation. Mutants in *NOXR*, encoding a regulatory subunit common to all NOX complexes, show, in addition to the phenotypes of *noxA*, drastically reduced virulence and less staining for superoxide when germinated on a glass surface. ROS production thus appears important for virulence. Mutants in *SOD1* and *SOD2*, predicted to encode Cu/Zn superoxide dismutases, show no obvious phenotypes, while preliminary evidence suggests the Fe/Mn type Sod3p is essential for normal development. We make some predictions for upstream kinases in the Hog1p cascade linking stress signals to gene expression. Stress response pathways may detect and respond to ROS, the level of which, in turn, depends on the balance between production and breakdown. (Poster # 529)

Reactive oxygen species- *Botrytis cinerea*'s friends or foes during host infection?

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Botrytis cinerea is a phytopathogenic ascomycete infecting a broad range of dicotyledonous plants. In the course of infection the necrotrophic fungus is exposed to reactive oxygen species (ROS) released by its host in the “oxidative burst”, an early plant defence reaction. But *B. cinerea* even produces ROS itself *in planta*. This raises the question how the pathogen senses and responds to the host defence reaction. Do ROS released by the plant harm the pathogen at all? Investigations on the AP-1 transcription factor Bap1 revealed its role as a pivotal regulator of ROS detoxification in axenic culture under exposure to H_2O_2 and menadione, an intracellular ROS generator. Macroarray analysis revealed 99 H_2O_2-induced Bap1 target genes. Besides other gene products, it controls transcription of several ROS degrading enzymes as well as of the thioredoxin and the glutaredoxin system, regulators of the cellular redox status. Interestingly, Bap1 is not essential for pathogenesis and its target genes are not expressed on the host 2 days post infection indicating a minor role for H_2O_2 degradation during infection. However, other redox- regulators like the SAPK BcSak1 or the Nox complex are essential for normal virulence. Therefore, we focus on the characterisation of factors involved in ROS signalling in order to connect different pathways and to elucidate their regulation. (Poster # 532)

A novel transmembrane protein is required for oxidative stress homeostasis and virulence in plant and animal fungal pathogens.

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Regulation of reactive oxygen species appears to be very important for pathogenic fungi during cell differentiation and pathogenesis. In this report we demonstrate that *Alternaria brassicicola* ATM1, a novel transmembrane protein, is necessary for tolerance to oxidative stress and plant pathogenesis. *ATM1* encodes a predicted hybrid membrane protein containing a single adenylation, six putative transmembrane, and FAD and NAD(P)-binding domains, and shows high sequence similarity to proteins found only in filamentous fungi including an animal pathogen *Aspergillus fumigatus*. Localization and gene expression analyses indicated that *ATM1* is associated with fungal Woronin body, a specialized peroxisome, and strongly expressed during conidiation and initial invasive growth in planta. *A. brassicicola ATM1*- deficient mutants exhibited abnormal conidiogenesis, accelerated loss of cell integrity of aged conidia, hypersensitivity to oxidative stress, and excessive oxidative burst of its own during conidiation and plant infection compared with wild-type strain. Virulence assay on green cabbage plant showed dramatically reduced virulence of the *Delta-atm1* mutants. Analysis of one of the *ATM1* homologs, *AtmA* in *A. fumigatus* revealed that the *ATM1* is functionally conserved in both plant and animal pathogenic fungi. Collectively, these results suggest that *ATM1* is likely to be involved in maintaining oxidative stress homeostasis during conidiation and pathogenesis. (Poster # 6)

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Integration of farnesol signaling in *Candida albicans*: importance of heterogeneous response in population for the promotion of level fitness in the face of oxidative stress.

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C. albicans is an opportunistic pathogen capable of causing a wide range of diseases, from mild mucosal diseases to life-threatening systemic infections. *C. albicans* frequently encounters high levels of oxygen reactive species (ROS) during interactions with other microorganisms and during infection of human tissues, and needs to reliably respond to these assaults to survive. Farnesol, a quorum sensing molecule produced by *C. albicans*, inhibits hyphal growth and protects cells against heat shock by inhibiting the Ras1/cAMP pathway (1). Previous data indicate that farnesol also protects against ROS stress (2). We questioned how farnesol signal is integrated by cells to enhance survival in response to ROS. We hypothesized that heterogeneity induced by farnesol promotes population level fitness in the face of oxidative stress. Mutant analyses were used to determine the respective contribution of the HOG MAP kinase and Ras1/cAMP pathways to farnesol signaling in response to ROS. The two pathways are involved in stress response and are altered by farnesol (1, 3). Then, we used flow cytometry sorting to analyse the resistance to ROS of subpopulations differentially sensitive to farnesol in yeast and hyphal inducing conditions.

1. Davis-Hanna et al. 2008. Mol Microbiol 67:47-62 2. Westwater et al. 2005. Eukaryot Cell 4:1654-61 3. Smith et al. 2004. Mol Biol Cell 15:4179-90 (Poster 194)

Cytoskeleton and Motors: Mike Plamann and Gero Steinberg

The *Aspergillus nidulans* kinesin-3 UncA motor moves vesicles along a subpopulation of microtubules

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The extremely polarized growth form of filamentous fungi imposes a huge challenge on the cellular transport machinery, as proteins and lipids required for hyphal extension need to be continuously transported to the growing tip. Recently, it was shown that endocytosis is also important for hyphal growth. Here we found that the *Aspergillus nidulans* kinesin-3 motor protein UncA transports vesicles along microtubules (MTs) and is required for fast hyphal extension. Most surprisingly, UncA-dependent vesicle movement occurred along a subpopulation of MTs. The MT cytoskeleton is characterized by its dynamic instability. In addition, MTs can be made up of different tubulin isoforms and of different post-translationally modified tubulins, such as acetylated or detyrosinated tubulins. GFP labelled UncA^{rigor} decorated a single MT, which remained intact during mitosis, while other cytoplasmic MTs were depolymerised. Mitotic spindles were not labelled with GFP-UncA^{rigor} but reacted with a specific antibody against tyrosinated alpha-tubulin. Hence UncA binds preferentially to detyrosinated MTs. In contrast, kinesin-1 (conventional kinesin, kinA) and kinesin-7 (KipA) did not show a preference for certain MTs. This is the first example for different MT subpopulations in filamentous fungi and the first example for the preference of a kinesin-3 motor for detyrosinated MTs.

Single dynein motors drive long-distance endosome transport in *Ustilago maydis*

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Organelle transport along microtubules, driven by the molecular motors kinesin and dynein, is a fundamental property of eukaryotic cells. An important example is the dynein-dependent retrograde motility of early endosomes in *U. maydis,* which is required for extended filamentous growth. In mammalians and fungi, dynein-based movements can span many micrometres. In vitro studies suggest that such long-distance movements depend on at least two cooperating dyneins, implying that multiple motors move an organelle in vivo. However, direct observation and quantification of dynein numbers in membrane trafficking of living cells has not taken place. Here we provide quantitative in vivo data which demonstrate that individual dynein motors are sufficient to move early endosomes over very long-distances in *U. maydis.* Using step-wise photobleaching and a nuclear pore component as an internal reference, we found that single dyneins undergo bi-directional motility over up to 40 micrometers along individual microtubules. Furthermore, we show that single dynein motors are capable of moving early endosomes over such long distances. Understanding the stochiometry of motors in long distance trafficking will provide new insight into membrane trafficking in fungi.

Functional Analysis of the Myosin II and Myosin V Homologs of *Aspergillus nidulans*

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We have investigated the roles of myosin II and myosin V in septation and tip growth in *Aspergillus nidulans*. Deletants of the myosin II homolog (here designated *myoB*) are viable, but colonies are wispy, hyphal branching is rare and septum formation is nearly absent. Tip growth, however, is slightly faster than in control *myoB*+ hyphae. A functional MYOB-GFP fusion protein localizes to nodes that coalesce at the site of septation. Deletants of the myosin V homolog (here designated *myoE*) are viable but colonies are compact. Tip growth is slower than in controls, branching occurs near the hyphal tip, hyphae are wider than in controls and localization of the vesicular snare SYNA reveals that the Spitzenkörper is absent. As in controls, SYNA localizes to the plasma membrane at the tip, and endocytic patches, as revealed by ABPA localization are localized to the cortex near the tip. Septa form, but may be incomplete, and are often nearer the tip than in controls. Movement of some SYNA containing vesicles appears slower than in controls. Observation of a functional MYOE-GFP fusion reveals that MYOE localizes to the Spitzenkörper and the general region near the tip, transiently to forming septa, and to moving dots in the cytoplasm that probably correspond to vesicles. Treatment with the anti-actin agent cytochalasin A causes the Spitzenkörper and MYOE to disperse, but some movement of MYOE dots continues. These data indicate that MYOB functions in septation but has little or no role in tip growth. MYOE plays a significant role in tip growth, perhaps by moving vesicles into the Spitzenkörper, but, by inference, it is not required for exocytosis or endocytosis at the growing tip. Supported by the DFG and NIH.

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Whole genome analysis of the *Aspergillus nidulans* kinesins.

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Kinesins are microtubule dependent motor proteins that are essential for many cellular functions such as intracellular transport and mitotic process. In the genome of *Aspergillus nidulans*, there are11 (putative) kinesin genes. We examined the role of these kinesins in the cell by deleting these genes and by observing fluorescently tagged products of these genes in living cells. One of the kinesin genes, *bim*C, is known to be essential for growth. We disrupted each one of remaining 10 kinesin genes. Although some of the disruptants exhibited relatively minor defects in growth, all of these disruptants were viable. This result indicated that the function of these kinesins are not mutually exclusive, rather, it is likely that the functions overlap. Live imaging of fluorescently labeled kinesins revealed the localizations of each kinesin in the course of cell growth. A group of kinesins exhibited rapid translocations along the cytoplasmic microtubules indicating this group of kinesins are involved in intracellular transport. Another group of kinesins localized in the nucleoplasm either throughout the cell cycle or in a cell cycle specific manner. Two of kinesins localized at the site of septum formation indicating a novel role of microtubule motor proteins in cytokinesis. Supported by NIH GM031837 and grant in aide from JSPS. (Poster # 264)

Exploring the Role of the C-terminal domain of Cytoplasmic Dynein Heavy Chain in *Neurospora crassa.*

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Cytoplasmic dynein is a large, microtubule-associated ATPase motor complex that facilitates minus-end-directed transport of various cargoes. Recent studies in the Plamann lab have focused on the largest subunit of the dynein motor, the dynein heavy chain (DHC), composed of six domains exhibiting varying degrees of homology to the AAA+ superfamily of ATPases. These domains are followed by a distinct C-terminal domain and together form a ring-like structure from which a microtubule-binding domain protrudes. Utilizing a genetic screening technique, point mutations in nearly all domains within the dynein motor head have been identified. We have now isolated revertants for a subset of these DHC mutants and have identified the respective intragenic suppressor mutations in >100 revertants. Most DHC mutations examined to date revert exclusively by intragenic suppression. However, approximately 75% of the AAA#6 and C-terminal domain DHC revertants contain extragenic suppressors, suggesting that AAA#6 and C-terminal domain mutations are readily bypassed by mutations in other genes. Identification and classification of extragenic mutations is currently underway using single nucleotide polymorphism (SNP) mapping, as well as comparison of motor activity amongst the DHC C-terminal mutants.

Microtubule cytoskeleton in the filamentous fungus *Ashbya gossypii*: organization and role in nuclear migration

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Nuclear migration is important for the normal growth and development of eukaryotes including filamentous fungi. In *Ashbya gossypii*, nuclear migration includes oscillatory movements along the hyphal growth axis and nuclear by-passing events. However, the molecular mechanisms underlying the different aspects of nuclear migration are still poorly understood and the intrinsic capacity of nuclei to sense cell polarity and move towards the hyphal tips has still to be determined.

We will present pictures and movies of the MT cytoskeleton in *A. gossypii* obtained by immunostaining and by visualizing GFP labeled microtubules. We will also show the roles of MT-binding proteins in building and maintaining the MT cytoskeleton and their importance for nuclear migration. The main focus will be on the MT plus-tip binding protein Bik1, the dynein and the dynactin subunit Jnm1. The phenotypes observed in the absence of those proteins will be presented as well as the localization of Bik1 and dynein. Modifying the dynamics of cytoplasmic MTs by either deleting Bik1 or dynein/dynactin or treating cells with MT-depolymerizing drugs strongly affected nuclear migration in *A. gossypii*. We could also show that in contrast to other filamentous fungi, cytoplasmic MTs do not directly contribute to hyphal growth.

Disassembly of septin filaments during transition from collar to ring-like structures

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Septins are conserved GTP-binding proteins essential for cytokinesis in animal and fungal cells. The filament-forming septins were discovered in the yeast *Saccharomyces cerevisiae* where they undergo a complex structural reorganization during budding growth. Septin filaments align in longitudinal orientation along the mother-bud axis and form an hourglass- or collar-like structure. During cytokinesis the septin collar is converted into two rings with circumferential orientation of filaments. The molecular mechanism of this dynamic structural transition is yet unknown. We could show that septin collar-to-ring transition in budding cells of the dimorphic fungus *Ustilago maydis* involves disassembly and reassembly of septin filaments. We used a chemical genetic approach to arrest dividing cells with a stable septin collar. We observed that at this stage the essential myosin light chain Cdc4 but not the FCH domain protein Cdc15 is associated with the septin collar. Inhibitor release results in instantaneous disassembly of the septin collar, while Cdc4 recruits Cdc15 from the cytoplasm to form the contractile actomyosin ring. Reassembly of septin filaments into a ring-like structure occurred upon constriction of the actomyosin ring. We propose that septin filaments exert a dual function in budding cells. Septin filaments in longitudinal orientation confer mechanical stability of the mother-bud neck while circumferential orientation of septin filaments is required for their function during cytokinesis. (Poster # 334)

A novel, microtubule dependent role for a formin in the filamentous fungus *Ashbya gossypii*.

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The assembly and organization of the actin cytoskeleton is fundamental for polar growth in many organisms including filamentous fungi. Key regulators of these processes are the Formin proteins. They contain characteristic sequence motifs termed formin homology domains that are important for subcellular localization and elongation of actin filaments. We show here that this is also true for the Formin from *Ashbya gossypii*. Mutation of *AgBNR2* results in an instable growth axis and frequent lyses of the tip. Since this phenotype is similar to hyphal growth after latrunculin treatment this suggests a role in actin regulation for *AgBNR2*. In agreement with this, we show that *Ag*Bnr2 is able to bind and polymerize actin. To our surprise a fusion of *Ag*Bnr2to GFP did not only localize to the tips of hyphae but also gave a punctuate pattern throughout the whole hyphae. We were able to show that these dots are identical with the spindle pole body (SPB) of the fungal nuclei. In addition we could identify a SPB-component as binding partner of *Ag*Bnr2 and we were able to map the binding motif in *Ag*Bnr2. Furthermore microtubule- binding and microtubule-stability assays suggest a direct role for *Ag*Bnr2 in regulation of microtubule dynamics in a way that is contrary to the microtubule stabilization known from mammalian formins. In conclusion our results suggest a dual role for the formin *Ag*Bnr2, with only one of its functions being related to actin and the other suggesting an involvement of *Ag*Bnr2 in the dynamics of nuclear migration via multiple microtubule interactions. (Poster # 259)

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Education and public outreach: Pat Pukkila and Pietro Spanu

How do we prepare future faculty?

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At Indiana University, a number of faculty have had the good fortune to participate in IU's Freshman Learning Project (FLP; http://www.iub.edu/~flp/), an intensive, two-week seminar in which faculty learn about a number of issues that affect student learning. Each participant also chooses a "bottleneck," which is a concept or thinking process that students find difficult. Participants then design classroom lessons to help students through those bottlenecks, practice these lessons on one another, and then implement them in their own courses.

I now lead a "Mentored Teaching" graduate seminar, which is meant as an FLP-like experience for graduate students. The course is based upon what Biology faculty found most valuable in their FLP experiences and on the advice of the teaching pros.

In this course, students: read about and discuss issues related to teaching in general and to science and science teaching; make field trips to observe effective teachers, particularly those engaged in "decoding the disciplines" methods of helping students learn to do disciplinary thinking; interview teachers of introductory courses about bottlenecks; choose a bottleneck and design a lesson to address it; practice the bottleneck lesson on peers; "road test" the lesson on a group of actual undergraduates; and write regular reflections on the readings, class meetings, and the bottleneck lessons.

In my poster and talk I will describe the course and also present topics and resources we have found the most useful so far. (Poster # 573)

DelsGate a robust deletion method used as a tool for undergraduate teaching in fungal genomics.

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A recently published method (Garcia-Pedrajas et al., FGB 2008) called DelsGate for the production of constructs for gene deletion in *Ustilago maydis* has been used as part of an upper division undergraduate laboratory course in applied biotechnology at The University of Georgia for the past three years. Students are each assigned a unique gene of research interest. Students are trained in accessing and downloading their gene locus from the genome sequence at the MIPS site MUMDB. They design primers for precise deletion of their gene, carry out the PCR reactions and check their construct for appropriate structure. Finally, they transform the fungus and analyze transformants for deletion of their gene of interest. Success rates are high due to the straight-forward and robust nature of the DelsGate approach. Students each compile a report of their experimentation including an analysis of potential gene function, genomic context of their gene and their deletion plan and experimental results and interpretations. The deletion mutants produced by class participants are fed back into the research lab and are further analyzed by laboratory personnel. Student evaluations suggest that the individual nature of this approach is appreciated for its real life research exposure. (Poster # 574)

Lessons learned from building a program for women in science

Joan W. Bennett, Rutgers University New Brunswick, NJ 08901.

After a long and happy career as an academic scientist, I unexpectedly started a new job in 2006 at Rutgers University with a half time appointment as a professor of plant biology and pathology and a half time appointment as an associate vice president. In the administrative role, my charge was to start an office for The Promotion of Women in Science, Engineering and Mathematics (WiSEM). Rutgers is a historically complex and geographically sprawling university with campuses in Camden, New Brunswick and Newark, NJ. Each campus is composed of multiple schools, colleges and centers. Women in SEM fields are found as students, postdoctoral researchers, research staff and faculty across the entire university, but in varying percentages. The numbers of women are lowest in the computer science, mathematics and engineering disciplines and highest in the social sciences. Although overt sexism is rarely a problem for contemporary SEM women, subtle biases and barriers remain. The talk will describe mentoring, leadership and other programs that the Rutgers WiSEM office has developed, with commentary on the advantages and disadvantages of training in fungal genetics to prepare one for meeting these challenges.

Neurospora Genetics and genomics summer research institute: an introduction to research.
Gloria E. Turner & Richard L. Weiss. University of California, Los Angeles

Phenotypic analysis of gene deletion strains was used to introduce college sophomores to the research process. When they entered the program, genetics, genomics and the model organism, *Neurospora crassa* were unknowns. At the completion they were well versed in these subjects as well as skilled in several basic microbiological laboratory techniques. We recruited students from UCLA and local Community Colleges that were participating in outreach programs for underrepresented minorities and first generation college students. A full-time nine-week program was developed focusing on the five assays that comprised the basic phenotypic analysis. These assays examined the fundamental biological consequences resulting from the loss of a single gene in a knockout (KO) mutant produced by the Neurospora PO1 project. The data generated by our program is transferred to the Broad Institute at Harvard/MIT. A data entry form designed to capture each mutant analysis aided in the information transfer. The students attended a scientific writing class, seminars given by prominent scientists, Excel and Power Point workshops as well as an introduction to NCBI. An independent research project was required of all students allowing them to generate a scientific question and research methodology. Each student performed their research and presented the results at the close of the program. These efforts have resulted in 900 KO phenotypes available on the *Neurospora crassa* database website at the Broad Institute. Equally important, several program participants are now pursuing masters and doctoral degrees in the sciences. (Poster # 575)

Undergraduate research in the state capital: Helping your state legislators understand and appreciate higher education.
Patricia J. Pukkila, University of North Carolina at Chapel Hill, NC 27599 USA

In 2001, we organized the first multi-campus undergraduate research symposium for a state legislature, and the symposium now involves all 16 campuses in our system. The purpose of the symposium was to convey the importance of original inquiry in undergraduate education and the ensuing benefits to citizens of the state. The event was well-attended and influential, and we have been invited back each year that the legislature is in its “long” session (2003, 2005, 2007, and 2009). Since that time, at least 12 additional states have organized similar symposia, with similar results. Key elements to a successful symposium include a university system-level legislative lobbyist to navigate scheduling and help raise legislator awareness, campus coordinators to select the student participants, involvement of a campus legislative lobbyist to help students connect with interested legislators and help the organizers evaluate the effectiveness of the event, office visits and/or introduction in chambers prior to the student poster session, and support from the campus chief academic officer. The event is surprisingly easy to coordinate, and has lasting value for state-university relations. Legislators deeply appreciate being shown the results of their support for the state’s universities, the relevance of student research and scholarship to current challenges faced by the state, and the gratitude of the students whose education has prepared them to address unsolved problems with confidence and to assume important roles as enlightened citizens and leaders. Supported by NSF EF-0412016

Plant pathology vs. Medical mycology: battle of the fungi.
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Although humans and plants are clearly different disease hosts, the study of fungal diseases in plants and animals share many more characteristics than are generally acknowledged. As one of the few departments to teach both Plant Pathology and Medical Mycology courses, we have a unique perspective. Plants and humans are similar in the taxa of fungal pathogens (Koch’s postulates!), modes of infection, and how pathogens travel through the organism. Plants and humans share broad categories of infections such as superficial diseases of the epidermis, traumatic implantation, and systemic pathogens. Conditions such as stress / weakened defense system or high heat and humidity can favor fungal growth in both groups. However, given the dissimilarity between the two hosts, the signs, symptoms, host defenses, and treatment methods differ greatly. Where is the disease triangle in medical mycology? Most fungal plant pathogens are specific to one host species, but can easily spread between individuals. In contrast, almost all fungal human diseases affect other mammals, but are typically not contagious. Human mycoses are typically more difficult to treat because of similarities between fungal and animal cells and their close phylogenetic relationship. The main purpose of this poster is to show that Plant Pathology and Medical Mycology are not so different after all, and, despite different terminology for similar processes and structures, both groups have much to learn from each other. (Poster # 576)

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Sex and development: Yin-Won Lee and Frances Trail

Coordination of fruit body formation and secondary metabolism in *Aspergillus nidulans*

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The homothallic filamentous ascomycete *A. nidulans* is able to form fruitbodies (cleistothecia) either by mating of two strains or by selfing in the absence of a partner. The three-dimensional *A. nidulans* cleistothecium is the most complicated structure this fungus is able to form. Differentiation and secondary metabolism are correlated processes in fungi that respond to various parameters including light, nutrients, aeration or pheromones. Our work on several proteins will be described, which are involved in the crosstalk between developmental regulation and secondary metabolism control in *Aspergillus nidulans.* They include the heterotrimeric *velvet* complex VelB/VeA/LaeA (Bayram et al., 2008, Science 320, 1504-1506.), where VeA bridges VelB to the nuclear master regulator of secondary metabolism LaeA, the eight subunit COP9 signalosome complex controlling protein turnover (Busch et al., 2007, Proc. Natl. Acad. Sci. USA 104, 8125-8130.) and the MAP kinase-related protein kinase ImeB (Bayram et al., 2009, Mol. Microbiol. 71, 1278-1295).

Microarray identification of genes differentially transcribed in strains of opposite mating types in *Podospora anserina*.

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Fertilization in the heterothallic *Podospora anserina* is controlled by the expression of the MAT1-1-1/FMR1 and MAT1-2-1/FPR1 transcriptional regulators in *MAT1-1/mat-* and *MAT1-2/mat+* partners, respectively. FMR1 and FPR1 candidate target genes were identified by comparing the transcriptomic profile of four strains: sexually proficient wild-type *mat-* and *mat+* strains, and *fmr1* and *fpr1* loss-of-function mutants. Differentially transcribed genes because of DNA polymorphism around the idiomorphs were eliminated from the analysis. One class of differentially transcribed genes consists of well-known mating-type target genes, as the pheromone receptor genes, the pheromone genes and the genes required for the pheromone processing. Another class contains numerous genes with no known function in fertilization. Their possible function during sexual cycle is currently investigated by deletion. These two classes amount to a total of 113 differentially transcribed genes that are controlled by activation and/or repression by either FMR1 or FPR1, or both of them, suggesting a very complex pattern of regulation. This work is funded by contract # ANR- 05-BLAN-0385-01 from the ANR. (Poster # 84)

B-regulated sexual development and the sugar transporter Sts1 in *Schizophyllum commune*.

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Mushroom development in the fungus *Schizophyllum commune* is normally the result of a sexual interaction between two individuals differing at what are termed the *A* and *B* mating type loci. *sts1* is a putative sugar transporter gene also implicated in the regulation of mushroom development. Null (delta-) mutant strains lacking functional copies of *sts1* displayed severely attenuated mushroom production. When delta-*sts1* strains were outcrossed, many of the delta- *sts1* null progeny displayed a “flat” phenotype, suggestive of an inappropriately-activated B mating type pathway. Activation of this pathway normally requires the mating of two haploid individuals with different B mating type specificities. Specific *B* loci encode both a G-protein coupled receptor (GPCR) and small lipopeptide pheromones that do not normally interact within self. We have investigated a possible link between the B mating type pathway and *sts1* by outcrossing our delta-*sts1* strain with a compatible strain containing an incomplete *B* mating type locus. Progeny from this cross were analyzed by both genetic and molecular approaches. The results of these analyses were consistent with the hypothesis that an intact *B* mating locus inherited from the delta-*sts1* parent was necessary in order for the “flat” phenotype to be displayed in a delta-*sts1* null mutant background. Our working model is that the activation of the B pathway by delta-*sts1* is the consequence of permitting activation of the GPCR by one of its' “self” pheromones. Reconstruction experiments involving the sequential addition of the GPCR and its' cognate pheromones to a *B* null, delta-*sts1* null strain will be performed to test this model. (Poster # 190)

Regulation of sex pheromone synthesis in Mucor-like fungi

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Zygomycetes use degradation products of carotenoids, especially trisporic acid and its precursors as extracellular communication system for recognising the complementary mating partner. Interestingly, the same communication system is used for the recognition of appropriate mucoralean hosts by the mycoparasitic zygomycete *Parasitella parasitica*. None of the partners can synthesize the active pheromone alone; the partners need to complement one another. The biological rationale of this system is that sexual or parasitic structures are exclusively differentiated in the presence of an appropriate partners.
We have identified the crucial enzymatic activities in the minus mating type of the model zygomycete *Mucor mucedo* from primary cleavage of carotene to the formation of trisporic acid. Regulation data for three of the corresponding genes will be presented. Whereas primary carotene cleavage by a carotene oxygenase is regulated predominantly at the transcriptional level, later steps, the oxidation from dihydrotrisporin to trisporin, and the oxidation from dihydromethyltrisporic acid to methyltrisporic acid, are considerably controlled by posttranscriptional and especially by posttranslational mechanisms. We also propose an enzymatic mechanism that allows the conversion between the B- and C- derivative series of trisporoids. The latter represents a first step towards understanding the vast metabolic diversity of trisporoids even in a single organism. Wetzel, J., et al. Eukaryotic Cell 8 (2009), 88-95.

Gene Regulation and Dikaryon Formation During Sexual Development of *Cryptococcus neoformans*

Emilia K. Kruzel and Christina M. Hull

Sexual development of the human fungal pathogen *Cryptococcus neoformans* occurs in five stages, including mate recognition, cell fusion, dikaryotic filament formation, basidium development, and spore formation. Spores are suspected infectious particles in cryptococcal disease. Although sexual development has been characterized visually, relatively little is known about the molecular events responsible for controlling each stage of development. To better understand how the process of sexual development occurs, we carried out a time-course microarray experiment spanning the time required for complete sexual development. RNA was prepared from crosses between opposite mating types (**a** and a) at time points representing each stage of development. To generate whole-genome expression profiles, RNA from each time point was assessed using gene expression microarrays. From these data, we identified eight temporally co-expressed groups of genes and have begun to assess the biological significance of this co-regulation. To begin to understand how dikaryon formation and subsequent growth take place, we are focusing on a group of genes that are induced during filamentation and repressed during the final stages of development (basidium formation, sporulation). We are characterizing the cis-regulatory elements upstream of these co-regulated genes to describe the transcriptional network responsible for the gross morphological changes observed during *C. neoformans* dikaryon formation.

Live-cell imaging reveals that arbuscular mycorrhizal fungi inherit and require hundreds of nuclei to survive.

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Biological inheritance involves the passing of traits from parents to offspring, with the genetic variation exhibited by individuals contributing to the evolution of the species. However, species evolution is less well understood for organisms undergoing asexual reproduction. One such group is represented by the arbuscular mycorrhizal (AM) fungi, ancient plant root symbionts, ubiquitous in most ecosystems that reproduce asexually via multinucleate spores. The mechanisms by which these spores are formed and the origin of the nuclear population provide an ideal model system to examine the genetic basis of evolution in coenocytic organisms. We use live real time cellular imaging to show the transmission of hundreds of nuclei into developing AM fungal spores. We find a significant positive relationship between spore size and nuclear content per spore for four AM fungal taxa as well as a surprising heterogeneity in nuclear content among spores. More importantly, we find that spores containing less than one hundred nuclei do not germinate. In contrast to all other known organisms, in which all somatic nuclei derive from a single progenitor nucleus, we discovered that AM fungi have a non-conventional system of heredity that has fascinating implications for the diversity of modes of evolution in eukaryotes. (Poster # 623)

DCL-1 colocalizes with other components of the MSUD machinery and is required for silencing

William G. Alexander, Namboori B. Raju, Hua Xiao, Thomas M. Hammond, Tony D. Perdue, Robert L. Metzenberg, Patricia J. Pukkila, and Patrick K.T. Shiu

In Neurospora, a gene present in an abnormal number of copies is usually a red flag for mischief. One way to deal with these potential intruders is by destroying their transcripts. Widely known as RNA interference (RNAi), this mechanism depends on the ‘‘dicing’’ of a double-stranded RNA intermediate into small-interfering RNA, which in turn guide the degradation of mRNA from the target gene. Here, we show that Meiotic Silencing by Unpaired DNA (MSUD), a mechanism that silences expression from unpaired DNA during meiosis, requires the dcl-1 (but not the dcl-2) gene for its function. This result suggests that MSUD operates in a similar manner to Quelling and other RNAi systems. We also show that DCL-1 colocalizes with SAD-1 (an RdRP), SAD-2, and SMS-2 (an Argonaute) in the perinuclear region. (Poster # 116)

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Analysis and regulation of carbon metabolism: Bernhard Seiboth and Ronald P. De Vries

Genes involved in controlling the response to carbon starvation in *Aspergillus nidulans*

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There is substantial evidence that a specific regulatory mechanism, distinct from carbon catabolite repression, exists for the response to carbon starvation in *Aspergillus nidulans*. Using production of extracellular proteases as a model, we have identified three genes that are involved in the response to carbon starvation but not carbon catabolite repression. Two of the genes encode non-catalytic hexokinase-like proteins (HxkC and HxkD) and the third gene product (XprG) belongs to a newly defined class of p53-like transcription factors. We used transcriptional profiling to show that XprG controls (directly or indirectly) the response of a large number of genes to carbon starvation. However, XprG does not appear to be involved in reverse carbon catabolite repression, in which carbon starvation blocks AreA-mediated activation of some genes required for nitrogen source utilisation. We investigated the interaction between *xprG* and genes involved in carbon catabolite repression. Analysis of mutants which lack hexose phosphorylating activity or the CreA repressor indicates that mutations which block glucose signalling or carbon catabolite repression lead to an increase in extracellular protease activity but do not abolish glucose repression. As the increase in extracellular protease activity in the *glkA4 frA2* and *creAD4* mutants was XprG-dependent it is possible that the effect of these genes is through modulation of XprG activity.

Role of hexokinases in carbon catabolite repression and induction in *Hypocrea jecorina*.

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In the genome sequence of *Hypocrea jecorina* two hexokinases encoding genes are found including one glucokinase (GLK1) and one hexokinase (HXK1). They are responsible for D-glucose respectively D-fructose phosphorylation and growth on these carbon sources. Deletion of both genes affects growth also on carbohydrates including D-xylose, L-arabinose or glycerol whose catabolism is independent of the catalytic function of the encoded hexokinases. The effect of the deletion of both hexokinase encoding genes on carbon catabolite (de)repression was analyzed by monitoring the expression of the cellulase *cbh1*, xylanase *xyn1*; and the *β*-galactosidase *bga1*. Carbon catabolite repression by D-glucose and D-fructose is retained in single deletion strains and only Delta-*glk1*Delta-*hxk1* strains are derepressed. The level of derepression in Delta-*glk1*Delta-*hxk1* strains was higher compared to the *cre1* (encoding the carbon catabolite repressor) mutant RutC30. Induction by specific carbohydrates differently influenced the expression of these genes: induction of *cbh1* by sophorose and *bga1* by D-galactose is impaired in Delta-*glk1*Delta-*hxk1* strains, whereas induction of *xyn1* by D-xylose is increased in all deletions strains being highest in a Delta-*glk1*Delta-*hxk1* strain. Our data suggest that the two hexokinases have additional non-enzymatic functions in carbon catabolism and signalling.

Mechanisms of regulation of amino acid transport and metabolism in phytopathogenic fungi

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How pathogenic fungi fulfill their nutritional needs during the interaction with their hosts remains poorly documented. Our studies focus on the amino-acid triggered regulation mechanisms required for the development of plant parasites. The gene encoding the monomeric GTPase Rheb, known to activate the kinase TOR, was silenced by RNAi in *Botrytis cinerea*. The implication of Rheb in the control of amino acid acquisition was appreciated with yeast complementation tests and the analysis of amino acid uptake/metabolism by reversed chromatography using HPLC. Mutants deleted of the gene encoding the bZIP transcription factor MetR, whose orthologue in *S. cerevisiae* controls the expression of the genes encoding the sulfate reduction pathway, were generated in *Magnaporthe grisea*. The putative MetR target genes were identified by testing the trophic requirements of the deleted strains, quantifying the intracellular amino acids and sulfur compounds by reversed chromatography and using transcriptomic approaches. The comparative analysis of the results obtained with both fungi –the *B. cinerea MetR* and *M. grisea Rheb* mutant strains being currently generated- will allow a better understanding of the mechanisms which control the transport and the metabolism of amino acids and sulfur compounds. (Poster # 371)

Carbon metabolism and *Cryptococcus neoformans* virulence

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Cryptococcus neoformans is an important fungal pathogen of immunocompromised individuals, with a close relative - *C. gattii* - emerging as a serious threat for the immunocompetent. During active infection, *C. neoformans* migrates to the brain and persists in the cerebrospinal fluid (CSF). Since CSF is a nutrient-limited environment, we sought to understand fungal carbon utilization in CSF. Prior studies have established the role of phosphoenolpyruvate carboxykinase (*PCK1*, links 3-carbon utilization with gluconeogenesis) in virulence using a inhalational mouse model. We evaluated a *pck1delta* mutant using a rabbit CSF model of virulence and found no defect in virulence for this mutant. Isocitrate lyase (*ICL1*), a key enzyme in the glyoxylate cycle (2-carbon utilization), was also previously examined by gene deletion and shown to have no impact on virulence in either the mouse or rabbit models of cryptococcal disease. In order to evaluate the use of various carbon sources by *C. neoformans* in the CSF environment, we created a *pck1delta/icl1delta* double mutant to evaluate the impairment of both 2- and 3- carbon utilization in a rabbit model of cryptococcosis. Furthermore, a pyruvate kinase (*PYK1*) mutant was made to examine the importance of glycolysis on growth and virulence in vivo. These studies serve to determine the relative roles of different carbon metabolism pathways in the persistence of *C. neoformans* in the CSF environment of the host. (Poster # 369)

Analysis of *Magnaporthe grisea* XlnR reveals significant differences in pentose catabolism from *Aspergillus niger*

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The ascomycete fungi *Aspergillus niger* and *Magnaporthe grisea* degrade dead or living plant matter respectively, to monomeric carbon sources. Xylose and arabinose are released from plant material by extracellular enzymes and catabolised through the pentose catabolic pathway (PCP). *M. grisea* has two lifestyles: it is a saprophyte and a plant pathogen. The PCP in *A. niger* is controlled by two transcriptional activators, AraR and XlnR. In *M. grisea*, XlnR may not only affect regulation of pentose utilisation for the saprophytic lifestyle, but may also influence the pathogenic lifestyle. Blast analysis identified a homologue for XlnR in *M. grisea*, but not for AraR. *M. grisea xlnR* was disrupted and the resulting strain was compared to the wild type with respect to growth on mono- and polysaccharides. Unlike in *A. niger*, disruption of *xlnR* abolished growth on xylose, but growth on arabinose was unaffected. This demonstrates that regulation of the PCP is differently organized in these two fungi. In addition, data will be presented on pathogenicity tests on barley and rice of the *M. grisea xlnR* disruptant compared to the wild type.

Effect of phoshoglucose isomerase deletion on celullase production in *Trichoderma reesei*.

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Trichoderma reseei (anamorph *Hypocrea jecorina*) stands out for its ability to degrade cellulose, hemicellulose and xylan. Majority of the cellulase genes are repressed by carbon catabolite repression and are induced by plant-derived polymers, such as cellulose, their degradation products, and by some oligosaccharides, as lactose and sophorose. RutC30 is a well-known hypercellulolytic mutant, known to have a truncated *cre1* gene, a mutation in the ER glucosidase II alpha subunit gene as well as a large deletion of 85 kb. We have generated a phosphoglucose isomerase (PGI) deletant of the strain RutC30 in order to block glycolysis and direct carbon flux to pentose phosphate pathway (PPP). The latter pathway is considered to be the main source of NADPH that is needed for many biosynthesis pathways. The *pgi1* mutant strains constructed were shown to lack PGI activity and to have an active PPP. These mutants were able to produce more cellulases than RutC30 in media with glucose as the carbon source. The same pattern was observed in media with glucose and fructose, where both glycolysis and PPP are active. However, in media with lactose and glucose (L+G) or with lactose and fructose (L+F), RutC30 produced more cellulases than the disruptants. And in media with L+F, RutC30 showed a 10-fold higher cellulose level as compared with media with L+G. The mutants were able to maintain cellulase production for a longer time during the cultivation as compared to RutC30. (Poster # 363)

Aconitase AcoA of *Aspergillus nidulans*, regulation at the atomic level?

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We study the propionate-degradation pathway of the filamentous fungus *A. nidulans*. In contrast to mammals, which use the methylmalonyl-CoA pathway for degradation, several microorganisms degrade propionate *via* the methylcitrate cycle (1). One of the key enzymes of this cycle is the methylaconitase, which we have shown to be the main aconitase (AcoA) of the citric acid cycle, too. This enzyme bears an interesting regulatory feature: the fully reduced 4Fe-4S cluster of this enzyme shows a constant ratio of aconitase and methylaconitase activity, whereby the oxidized 3Fe-4S cluster only shows methylaconitase activity. Therefore, the ratio of both activities indicates the oxidative state of the enzyme AcoA. We tested wild type cultures of *A. nidulans*, grown on different carbon sources for both enzymes. The different ratios implies the relevance of this regulatory mechanism *in vivo*. 1.Tabuchi, T., and Hara, S. (1974) Production of 2-methylisocitric acid from n-Paraffins (Poster # 358)

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Mechanisms of action of transcriptional regulators involved in fatty acid catabolism.

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FarA, FarB and ScfA are Zn(II)2Cys6 transcription factors which upregulate genes required for growth on fatty acids in *Aspergillus nidulans*. FarA and FarB are highly conserved and bind to CCGAGG core sequences in the promoters of their target genes. My project focuses on investigating how they work together to control gene expression. *farA* overexpression strains show an increase in reporter gene activity in the presence of oleate but a decrease is seen when *farB* is overexpressed. This led to a proposed model in which a FarA homodimer activates genes when a long-chain fatty acid is present, but a FarA-FarB heterodimer (with ScfA potentially interacting) increases expression during growth on short-chain fatty acids. Protein interactions are being determined using yeast two hybrid analysis. In the heterodimer, FarB might receive the short-chain fatty acid signal, while FarA provides the activation ability. FarB-FarA fusion proteins were created in which different putative activation domains of FarA were inserted into the C-terminus of FarB, creating FarB proteins that have activation capability. These proteins were tested in a *farA?* background with growth significantly increased compared to wildtype *farB*. The activation ability of FarA may be its main contribution to short-chain fatty acid dependent expression, but it may also have other roles. (Poster # 143)

CONCURRENT SESSIONS IV

Zygomycetes and chytrids: Mat Fisher and Santiago Torres

Sex locus and virulence of zygomycetes and microsporidia, the basal fungi

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Sexual reproduction of fungi is orchestrated by a delimited chromosomal region known as the mating type or *sex* locus. This locus is also involved in virulence in some pathogenic fungi. In this study we defined the *sex* loci of two different basal fungal lineages, zygomycetes and microsporidia. A previous study showed that, in the zygomycete *Phycomyces blakesleeanus*, the *sex* locus encodes a high mobility group (HMG) transcription factor and is flanked by genes encoding a triose phosphate transporter (TPT) and an RNA helicase. We found that microsynteny of the *sex* locus [TPT, HMG, RNA helicase] is highly conserved across zygomycetous fungi including *Rhizopus oryzae* and *Mucor circinelloides* and excluding other fungal lineages (Basidiomycota, Ascomycota, and Chytridiomycota). Human pathogenic zygomycetes cause a life threatening infection, mucormycosis. We found that minus mating type and large spore producing isolates are more virulent in *Galleria mellonella,* providing a possible link between the *sex* locus and pathogenicity of mucormycosis.
We also found that three microsporidians, *Encephalitozoon cuniculi*, *Enterocytozoon bieneusi*, and *Antonospora locustae*, harbor a similar syntenic *sex* locus. *R. oryzae* and *E. cuniculi* share a higher level of genomic architectural similarity based on relaxed synteny analysis compared to other fungal lineages, providing evidence these obligate intracellular pathogens are true fungi evolutionarily related to the Zygomycota. The presence of a *sex* related locus in microsporidian genomes suggests these obligate pathogens may have an extant sexual cycle and studies to address this and its possible relationship to pathogenesis are in progress.

Annotation and analysis of zygomycetes: *Phycomyces blakesleeanus* and *Mucor circinelloides*

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Despite the increasing number of fungal genome sequencing projects, the phylogenetic diversity of the sequencing targets is extremely limited. Two zygomycetes - *Mucor circinelloides* and *Phycomyces blakesleeanus*– were recently sequenced at JGI and represent the poorly sampled branch of the fungal tree. *Mucor circinelloides* is a candidate feedstock for biodiesel because it can accumulate large amounts of suitable lipids. The photoresponsive zygomycete *Phycomyces blakesleeanus* is an experimentally tractable model system for elucidating the signalling pathways underlying photoregulation. These genomes show significant variation in genome sizes and gene content. Sequenced cDNAs from mycelia grown with and without light not only provided support for predicted gene models but also suggested the genes with potentially distinct expression patterns both species: light- and dark-specific. Together with *Rhyzopus oryza*, *M. circinelloides* and *P. blakesleeanus* are the only three zygomycetes with a sequenced genome, providing an opportunity to discover genes that are specific to or missing from zygomycetes, and gene families that are expanded or contracted in zygomycetes relative to the much better-sampled ascomycetes and basidiomycetes.

Rapid expansion the emerging fungal disease chytridiomycosis into declining and healthy amphibian populations

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The fungal disease chytridiomycosis, caused by *Batrachochytrium dendrobatidis*, is enigmatic because it occurs in both healthy and declining amphibian populations. This distribution has fueled debate concerning whether, in sites where it has recently been found, the pathogen was introduced or is endemic. In this study, we addressed the molecular population genetics of a global collection of fungal strains from both declining and healthy populations using DNA sequence variation at 17 loci. The pathogen has extremely low DNA polymorphism and an excess of heterozygosity at multiple loci, consistent with a primarily clonal mode of reproduction. Nonetheless, a high diversity of multilocus diploid genotypes was observed, some of which we hypothesize could be explained by loss of heterozygosity through mitotic recombination. None of the geographic or host populations possessed the genetic signatures of a source population, though strains from temperate North America and Europe had slightly higher diversity than tropical populations. One strain isolated from a bullfrog contained as much genetic diversity as the entire global sample. These data are largely consistent with the fungus as a novel pathogen undergoing a rapid and recent range expansion. (Poster #398)

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Mapping evolving *Batrachochytrium* lineages.

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The chytrid fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is now recognised as a proximate driver of amphibian declines. Population genetics and histological examinations of museum specimens indicate that *Bd* has only emerged in the latter half of the 20th century, and is rapidly spreading to infect new regions and naïve populations and species. We have developed an online tool, www.spatialepidemiology.net/Bd-maps that is used by a widely-collaborative group of scientists to map the global epidemiology of *Bd*. I demonstrate how this dataset has the potential to predict the trajectory of disease by identifying 'at risk' areas of the globe, and by 'tracking' *Bd* genotypes using spatial epidemiological techniques. Recent data show that *Bd* isolates can be profiled by their genotypic and proteomic characteristics, as well as by the size of their sporangia. *Bd* genotypic and phenotypic distance matrices are significantly correlated, showing that less-related isolates are more biologically unique. Mass spectrometry has identified a set of candidate genes associated with inter-isolate variation. Our data show that, in spite of its rapid global emergence, *Bd* lineages are not identical and differ in several important characters that are linked to virulence. We argue that future studies need to clarify the mechanism(s) and rate at which *Bd* is evolving, and to map the spread of these lineages in order to ascertain the impact that this variation has on the host-pathogen dynamic.

Genomic analysis of the basal lineage fungus *Rhizopus oryzae* reveals a whole-genome duplication

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Rhizopus oryzae is the primary cause of mucormycosis, an emerging life-threatening infection characterized by rapid angioinvasive growth with an overall mortality rate that exceeds 50%. As a representative from the fungal basal lineages, *R. oryzae* has also been used as a model to study complex life cycles with multicellular stages.

Here we report the genome sequence of *R. oryzae* strain 99-880, which is a clinical isolate from a fatal case of mucormycosis. The highly repetitive 45.3 Mb genome assembly contains 17,467 predicted protein-coding genes, many of which are paralogous pairs. The order and genomic arrangement of the duplicated gene pairs and their common phylogenetic origin provide evidence for an ancestral whole-genome duplication (WGD). The WGD results in the duplication of nearly all subunits of the protein complexes associated with oxidative phosphorylation, the vesicular ATPase, and the ubiquitin–proteasome systems. We also observe the expansion of gene families related to cell growth, signal transduction, as well as secreted aspartic protease and subtilase protein families, which are known fungal virulence factors.

The post-WGD retention of protein complexes and gene family expansions may contribute to the rapid disease development of *R. oryzae* infections.

Expanded families of cell-wall synthesis enzymes, essential for fungal cell integrity yet absent in mammalian hosts, reveal potential targets for novel and R. oryzae-specific diagnostic and therapeutic treatments.

As an early diverging fungus, *R. oryzae* shares a higher number of ancestral genes with the metazoa than dikaryotic fungi (ascomycetes and basidiomycetes), demonstrating its utility for the study of eukaryotic regulation and developmental processes that may not be addressed in dikaryotic fungal model systems.

Regulation of photocarotenogenesis via proteolysis-independent ubiquitylation in the zygomycete *Mucor circinelloides*

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Protein ubiquitylation plays a major role in the regulation of cellular processes through proteasome-dependent degradation of proteins, although it has become increasingly clear that it is also involved in mechanisms other than simple targeting to the proteasome. In *Mucor*, blue light regulates carotene biosynthesis. This response is controlled by two independent regulatory pathways, with *crgA* and *mcwc- 1c* as the corresponding key genes. CrgA shows characteristics of ubiquitin ligases and represses carotenogenesis, whereas *mcwc-1c* is a *white collar 1*-like gene required for light induction. The function of *crgA* in carotenogenesis is mediated by *mcwc-1b*, another *white collar 1*-like gene that acts as a carotenogenesis activator. CrgA controls MCWC-1b function by proteolysis-independent mono- and di-ubiquitylation, which results in MCWC-1b inactivation. This is the first description of this regulatory mechanism in filamentous fungi, and suggests that it could be more widespread than previously thought. A proteomic analysis revealed that CrgA regulates the levels of a number of proteins both through MCWC-1b and independently of it, suggesting that MCWC-1b is not the sole CrgA target. Moreover, these results suggest that proteolysis-independent ubiquitylation is used in *Mucor* to control other cellular processes, in addition to carotenogenesis. (Poster # 179)

Environmental DNA combined with fluorescent *in situ* hybridisation reveals a missing link in the fungal tree of life.

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Chytridiomycete fungi are important microbial components of aquatic environments. However, due to a reliance on culture based analysis, the evolutionary complexity of the 'chytrids' has been largely underestimated. Environmental gene library analyses of aquatic environments has recently demonstrated a highly novel form of deep branching 'fungi', the environmental diversity and complexity of which indicate that these uncharacterized-organisms are an important component of aquatic ecosystems. Here, we use a combination of environmental PCR and fluorescent *in situ* hybridisation (FISH) to elucidate the complexity and evolutionary history of these novel 'fungi'. PCR amplification and DNA sequence analysis extending through the 18S, 5.8S and 28S rRNA encoding genes, allowed improved phylogenetic analysis using complex models. The development of FISH probes reveals our target group to be a picoeukaryote of 4-5 micrometers in diameter and ubiquitous within local freshwater sites. Cells were morphologically similar and were often visualised in high numbers within structures we suggest to be sporangia. Interestingly, cell wall staining reveals our target organisms to be lacking in the characteristic fungal cell wall, unlike known 'chytrid' groups. This, in combination with our phylogenetic data, suggests this highly diverse group may be a missing link in the fungal tree of life. (Poster # 427)

Elongation factor 2 phylogeny of *Olpidium* and its implications for early fungal evolution.

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Olpidium brassicae is one of the ~1000 fungal species that retained the unicellular, flagellated, aquatic habit that was present in the ancestor of all true fungi. Surprisingly, phylogenies from rDNA and RPB1 (James et al. 2006) placed *Olpidium* nearest terrestrial zygomycetes instead of among other unicellular water molds, albeit without bootstrap support. To challenge this result, we sequenced and analyzed the elongation factor 2 gene from zygomycetes and chytrids including two *Olpidium* species. Maximum likelihood analysis showed that the *Olpidium* species form a monophyletic clade with terrestrial entomophthoralean and harpellalean zygomycetes, but still without bootstrap support. Shimodaira-Hasegawa tests rejected the placement of *Olpidium* within the Chytridiomycota or Blastocladiomycota clades, however, placements basal to the Chytridiomycota or within the zygomycete clades were not significantly worse. *Olpidium* may represent one of the earliest diverging of all chytrid lines, or it could be the closest living aquatic relative of a terrestrial zygomycete lineage. Sequences from additional loci will help decide between these possibilities and either way, *Olpidium*'fs characters will provide basic information about features of early evolving fungi. (Poster # 412)

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Cool tools for fungal biology: Gary Foster and Steve Osmani

Transforming mushroom biology

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Whilst the last few years have seen a dramatic increase in our understanding of the biology of Ascomycete fungi, our knowledge of other groups has not moved ahead as rapidly. This difference has been highlighted by the speed with which genome sequence data has been exploited in model species such as *Aspergillus* and *Neurospora*, whilst in model Basidiomycetes such as *Coprinopsis* such data may be seen as having comparatively little impact, so far. We believe this is largely due to a lack of tools for easy manipulation of such species. Here we report on progress in our understanding of how to manipulate basidiomycetes including optimisation of transformation systems, selection markers, expression systems and silencing systems. Whilst much of this is centres on development of systems for *Coprinopsis cinerea*, our studies have shown many of these are also applicable to commercially important species such as the edible mushroom *Agaricus bisporus* or those producing important pharmaceuticals such as *Clitopilus passeckerianus*.

Novel tools for gene manipulations and a luciferase-based reporter system in *Neurospora crassa* reveal detailed real-time dynamics of *frq* /FRQ oscillations and uncovers new period mutants

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In every genetic system, the availability of tools for genetic recombineering and the availability of dependable and sensitive real time reporters are essential to progress. Furthermore, development of tools in one system often provides the springboard for dissemination of similar tools within the fungal genetics community. In *Neurospora crassa* the ability to perform very high (<98%) efficiency homologous gene replacements has fostered the development of a number of different knock-out, knock-in, and gene tagging strategies that have vastly expanded the repertoire of tools available. In addition, the recent development of luciferase as a real time reporter has greatly facilitated monitoring gene and protein expression and regulation. Application of these novel tools to the study of rhythms in *Neurospora* provides a revealing list of case studies. The *Neurospora* circadian oscillator is composed of a transcriptional translational negative feedback loop, where the phosphoprotein Frequency (FRQ) inhibits its own expression by affecting the activity of the white collar transcriptional complex (WCC), giving as a result robust oscillations in FRQ protein and message levels. These rhythms can be indirectly followed by the overt circadian regulation on spore formation (conidial banding) although technical limitations restrict the routine molecular analysis of *frq* message and protein levels to no more than 2-3 days. To overcome this and other limitations we have developed a fully-codon optimized luciferase reporter system for *Neurospora crassa*. Thus, by putting this real-time reporter under the control of the entire *frq* promoter, or discrete regions containing circadian elements, rhythms in *frq* transcription can be easily tracked for over a week. Moreover, by generating FRQ-LUC translational fusion strains, rhythms in FRQ protein can be followed in a semiquantitative manner. We have combined this bioluminescence-based system with a variety of tools for gene manipulation generated in association with the *Neurospora* Genome Project, such that different deletions strains can be easily analyzed for circadian molecular phenotypes. These facilitate reporter and regulatable promoter knock-ins, N- and C- terminal epitope tagging with a variety of tags, and strains designed to facilitate specific screens. Moreover, the circadian oscillator of strains with severe growth defects or no defined "banding" can now be revealed, an otherwise daunting task, when performed by classic western/northern approaches. In addition to genetic analyses, pharmacological perturbations can be easily performed, altogether revealing unexpected molecular details of the clockworks.

Manipulating living fungal cells with light

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Optical traps (commonly known as laser or optical tweezers) allow the manipulation of inert and biological microscopic objects solely by using light. Since their invention in 1986, they have been used as experimental tools in a wide variety of applications in the life sciences. We are using optical traps to manipulate and interrogate living fungal cells. In my talk I will show how single beam and holographic optical traps are being employed to investigate growth, cell-cell communication, mechanosensing and the biophysical properties of fungal cells (Wright et al. 2007; Burnham et al. 2007). Burnham DR, Wright GD, Read ND, McGloin D (2007) Holographic and single beam optical manipulation of hyphal growth in filamentous fungi. J Optics A: Pure Appl Optics 9**:** S172-S179. Wright GD, Arlt J, Poon WCK, Read ND (2007) Optical tweezer micromanipulation of filamentous fungi. Fungal Genet Biol 44: 1-13.

Comprehensive methods to inactivate, visualize and purify all fungal proteins.
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To apply systems level biological approaches within the filamentous fungi, tools and methodologies need to be developed to efficiently inactivate, visualize and purify proteins on a large scale. In order to carryout studies of the dynamic behavior of all components of macromolecular nuclear structures during *Aspergillus nidulans* mitosis we have developed such methodologies. This necessity arose as we are studying nuclear pore complexes (NPCs) and the nucleolus, two massive yet dynamic structures composed of hundreds of interacting proteins. Importantly, both of these nuclear structures are dramatically disassembled then reassembled during each nuclear division cycle. These massive reorganizations are regulated on a time scale of minutes by reversible phosphorylation in manners yet to be determined. Methods we have developed to study the regulation of NPC and nucleolar disassembly-reassembly will be described that enable systems level analysis using: (a) targeted gene deletions and phenotypic analysis of both essential and non-essential genes, (b) 5D analysis of the dynamic subcellular location of proteins using endogenous fluorescent tags, (c) endogenous affinity tagging for one step purification and LC/MS/MS analysis to define dynamic protein-protein interactomes and associated post translational modifications. Although developed for *A. nidulans*, many of the approaches should be applicable for use in other filamentous fungi.

Exploring fungal biology using light-, electron- and spectro-microscopy.
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For over a century, studies of the growth of mycelia and of individual hyphae in culture using microscopy, has generated and reinforced the paradigm that most of the really important events are at extending tips. In part, this has been due to the disparity in spatial resolution between microscopy and chemical analyses. Chemical analyses had been limited to a few targets at high spatial resolution (e.g. immuno-based and chemical- specific probes, monitored using microscopy) or high chemical resolution of bulk samples (e.g. mass spectroscopy). However, fungal hyphae change in structure and function within nanometers to microns, and vary between individual hyphae in a common environment. Ongoing development of high spatial resolution spectromicroscopic methods has bridged this gap. Here, we show preliminary correlative studies using light and electron microscopy and high spatial resolution chemical analyses: Fourier transform infrared spectroscopy and X-ray fluorescence spectroscopy (both using brilliant synchrotron light sources); Raman and SERS spectroscopy; imaging mass spec using laser micro-ablation. Together, we are using these techniques to better understand the roles of tip and basal hyphal regions in saprotrophs, pathogens, and endophytes. Using complementary methods has allowed us to begin to refine our ideas of how fungi interact with their physical and biological environments. Supported by NSERC (KG and SK), CIHR (KG), CIHR-RPP (SK) (Poster # 296)

The *Aspergillus* Genome Database (AspGD), a curated database of *Aspergillus* gene, protein, and genomic sequence information for the fungal research community.
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We have recently obtained funding to create a resource for the *Aspergillus* research community, AspGD, which will be a multispecies genomics database modeled on the *Candida* and *Saccharyomyces* Genome Databases. Our goal is to facilitate research on Aspergilli and on other medically and economically important fungal pathogens by providing a first-stop reference for *Aspergillus* genomics and molecular biology, with up-to-date, high-quality, curated scientific information and web- based research tools. We will implement an optimized annotation pipeline across all *Aspergillus* genomes, to maintain a set of current and consistent gene boundary annotations, and will incorporate links between genomes based on orthology and synteny data. Gene function, localization, and mutant phenotype annotations will be manually curated from the *Aspergillus* literature. We will provide web-based tools for sequence visualization and retrieval, and for analysis of sequence information and gene product annotations. All of the data in AspGD will be freely available to the public from http://www.aspgd.org/. In addition, we will maintain a colleague registry by which *Aspergillus* research community members may share contact information and research interests, to facilitate collaboration. We strive to be responsive to the needs of the research community, and we welcome your feedback and suggestions.
AspGD curators may be reached by email at aspergillus-curator@genome.stanford.edu AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH. (Poster # 28)

Development of fluorescent protein-based biosensors for Ca^{2+} and pH to monitor physiological changes during *Arabidopsis thaliana-Fusarium oxysporum* interactions

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Both calcium and pH are important signal molecules that participate in the perception of many different types of external stimuli and control growth of both the plant and the pathogen. Changes in cytosolic free calcium ($[Ca^{2+}]_C$) can be evoked by various external stimuli and lead to physiological and/or developmental changes of the cell. The pH sensing-response signaling pathway also affects the activity of pathogenicity factors secreted by certain pathogens. To monitor real-time physiological changes during plant-pathogen interactions under different environmental conditions, we employed fluorescent Fluorescence Resonance Energy Transfer (FRET)-based Ca^{2+}sensors (Cameleon) and a pH sensitive biosensor. We successfully transformed two fungi, *Fusarium oxysporum*, *Magnaporthe oryzae*, and *Arabidopsis thaliana* with this FRET sensor and the pH biosensor to study Ca^{2+}/pH dynamics during their interactions. Time-lapse confocal imaging confirmed a tip high calcium gradient and pH changes in both fungal species. In addition, calcium responses in relation to key events in fungal growth such as branching, septum formation and cell-cell contact were observed. We also observed Ca^{2+}/pH changes during fungal growth on plants and response to other substrates. The combination of molecular genetics and molecular cytology tools with these biosensor tools should help us dissect the genetic, cellular, and biochemical basis of pathogen- plant interactions at the cellular and tissue levels. (Poster # 559)

Progress under the Fungal Genome Initiative : Sequencing and comparative analysis of fungal genomes

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The Fungal Genome Initiative was launched in 2002 to generate genomic resources including fungal genome sequences, annotations, and analytic tools to support research on organisms across the fungal kingdom. To date the FGI has released genome sequences for 57 fungi, including fungi from all four major branches of the fungal tree. In addition, 9 approved fungi are currently in the sequencing queue. A primary focus of the FGI has been to promote comparative studies by sequencing clusters of related fungi and phenotypic variants of species to help interpret the biology of key models or pathogens. Recently, major sequencing efforts have targeted clusters of species as well as multiple strains of human pathogenic fungi. These species, including Candida, dermatophytes, and the dimorphic pathogens (*Coccidioides*, *Paracoccidioides*, *Blastomyces* and *Histoplasma*) have been selected for comparative analysis to identify genomic attributes that could contribute to the pathogenesis of these highly infectious fungi. We will describe recent progress on analysis of these genomes as well as new tools and web sites to help make these comparative data most useful to the community. We will also address how we are applying new sequencing technologies to produce new genome assemblies and look at genome-wide variation. (Poster # 108)

Nitrogen regulation networks: Bettina Tudzynski and Meryl Davis

The coordinated cellular response to nitrogen availability in *A. nidulans*.

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Perception of the quality and quantity of nitrogen available to the cell and the coordinated response in A. nidulans involves multiple regulatory and signalling mechanisms. Our current research is focused on three areas: The post-translational modification and intracellular localisation of AreA and other transcription factors involved in regulating nitrogen metabolism. The molecular mechanisms involved in regulating mRNA stability which for a large number of genes acts in tandem with transcriptional regulation to determine appropriate levels of gene expression in response to changes in nitrogen availability and quality. Global assessment of gene regulation, utilising proteomics, transcriptomics and high-throughput transcriptome sequencing. Our aim is to monitor the full complexity of the regulatory response to changes in the nitrogen regime. We shall present our recent data which highlights the interrelationships between nitrogen and other regulatory responses. (Poster # 149)

Deletion of the *Aspergillus nidulans* nitrogen regulatory gene *areB* reveals pleiotropic phenotypes.

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The *Aspergillus nidulans* transcription activator AreA is a key regulator of nitrogen metabolic gene expression. AreA transcription capacity is highly regulated in response to nitrogen nutrient quality and/or availability by autogenous transcriptional activation, differential *areA* mRNA stability, interaction with the NmrA corepressor and the TamA coactivator, and regulated AreA nuclear export. AreA contains a C-terminal GATA zinc finger DNA binding domain that is highly conserved in *areA* homologues in other filamentous fungi. In *A. nidulans*, another GATA factor AreB containing an N-terminal GATA domain and a C-terminal leucine zipper domain has been implicated in nitrogen regulation. Evidence that AreB acts in nitrogen regulation was provided by gain-of-function mutations in *areB*. AreB and NreB, the *Penicillium chrysogenum* ortholog, are highly conserved and NreB overexpression suggested that NreB acts negatively possibly by competing with AreA for DNA binding. However, AreB and NreB are likely orthologous to the *Neurospora crassa* sexual development regulator Asd4, which does not function in nitrogen regulation.
In order to investigate the role of AreB in nitrogen regulation, we determined the *areB* deletion phenotype. Gene replacement mutations in *areB* or *nreB* were not previously reported. AreB antagonizes AreA activation and functions in growth, asexual development and conidial germination but not sexual development. Overexpression of AreB prevents AreA-dependent gene expression and confers severe growth inhibition. Both the DNA-binding domain and the leucine zipper of AreB are required for AreB functions. These data indicate that AreB has a wide domain of action including but not limited to nitrogen metabolic genes.

Integration of nitrate assimilation into the nitrogen metabolic network of *Aspergillus nidulans*.

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Nitrate, one of the most abundant nitrogen compounds in soil, is metabolized by microorganisms and plants. In most of these organisms nitrate is only assimilated when metabolically less costly, and thus preferred, nitrogen sources such as ammonium or glutamine become limited and when nitrate or nitrite is present as inducing compound. The regulatory network responsible for the integration of the activating and repressing nutritional signals has been thoroughly studied in *Aspergillus nidulans*. The transcription factors AreA and NirA are required for the induction process in which AreA is positively responding to nitrogen limitation and NirA is positively responding to the inducers. Under nitrogen repressing conditions (high levels of preferred nitrogen sources) AreA function is down-regulated by a variety of transcriptional and post-transcriptional mechanisms. Under nitrogen limiting conditions AreA mediates histone acetylation and concomitant opening of chromatin, thereby setting the stage for NirA action. Intracellular nitrate acts on NirA by disrupting its interaction with the nuclear export machinery, resulting in nuclear accumulation of NirA and AreA-facilitated DNA binding in promoters of NirA target genes. This synergistic interaction leads to nucleosome hyperacetylation, complete chromatin remodelling and transcriptional activation of the assimilatory genes. Continuous nitrate assimilation leads to a replenishment of the intracellular glutamine pool which partially inactivates AreA and this feed-back loop leads to downregulation of the assimilatory activity. Comparing the nitrate response with nitrogen limitation in a transcriptome analysis reveals that the transcriptional response to nitrate is complex and encompasses both NirA-dependent and NirA-independent regulatory events.

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Exploring interactions among ammonium transporters of fungi.

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Fungal proteins that sense carbon and nitrogen availability interact with conserved signaling pathways to regulate mating and the transition from budding to filamentous growth. Ammonium transporters (e.g., Mep1,2,3 from *Saccharomyces cerevisiae* and Ump1 and Ump2 from the corn smut, *Ustilago maydis*) are important for uptake of ammonium as a nitrogen source. Moreover, Mep2 and Ump2 can sense low ammonium availability and transmit this signal to trigger the dimorphic switch. Interestingly, we have found that *U. maydis* cells over-expressing the *ump2* gene grow filamentously in carbon- and nitrogen-replete conditions. Similar findings in *Candida albicans* and *S. cerevisiae* suggest that ammonium limitation per se might not be required for induction of the dimorphic switch in response to ammonium. We used split ubiquitin to further explore possible interactions among Ump1, Ump2, and their putative signaling pathway(s). The results suggest that *U. maydis* transporters Ump1 and Ump2 physically interact with themselves and with each other. Moreover, they appear to have weak interaction with a Rho1 homologue, thus suggesting an avenue for the signaling cascade. We are also investigaing the ammonium transporter homologues in another smut fungus, *Microbotryum violaceum*. One of the genes, *mepA*, complemented the transport function in the *S. cerevisiae mep1,2,3* triple deletion mutant; however, it was not able to complement the filamentation defect on low ammonium for either this mutant or the *ump2* mutant of *U. maydis*. Predicted amino acid sequence of a second homologue, MepC, reveals various levels of similarity to other well studied ammonium transporters and is currently under investigation to evaluate its possible roles in transport and filamentation. (Poster # 182)

Nitrogen regulation in *Aspergillus nidulans* and *Fusarium fujikuroi*: the same players-different roles?

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Most of our knowledge on the molecular mechanism of AreA-dependent nitrogen regulation derives from studies in *A. nidulans*. In this fungus, AreA activity is controlled by multiple mechanisms including regulated *areA* transcript levels and regulated AreA nuclear export. A major player in this network is NmrA which negatively affects AreA activity by binding it when rich nitrogen sources are available. Deletion of the *nmrA* gene resulted in significant de-repression of AreA target genes under nitrogen sufficient conditions while *nmrA* overexpression prevents AreA function irrespective of the nitrogen status. Recently it has been shown that the bZIP transcription factor MeaB activates *nmrA* expression, and that a conserved element in the promoter of NmrA, TTGCACCAT, is bound by MeaB in vitro. Expression of *meaB* was not strongly regulated suggesting that transcriptional activation by MeaB is modulated by the nitrogen status. In contrast, in *F. fujikuroi*, the NMR homologue was shown to play only a minor role in nitrogen regulation, although Y2H experiments clearly demonstrated that it binds AreA as in *A. nidulans*. *MeaB* transcript level and transcript size are strictly regulated by nitrogen availability in an AreA-dependent manner, and MeaB seems not to be essential for *nmrA* expression as shown by reporter gene analysis. On the other hand, gutamine synthetase (GS) plays an important role in regulating the expression of nitrogen-dependent genes in contrast to *A. nidulans*. Therefore, we postulate that *F. fujikuroi* must contain additional regulatory proteins that affect AreA activity in a more efficient way than Nmr and MeaB, and that these additional proteins may be involved in protein interactions with AreA and/or GS in response to intracellular nitrogen status.

Trehalose-6-phosphate synthase integrates metabolic control and fungal virulence in *Magnaporthe oryzae* via a novel NADP(H)-dependent genetic switch.

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Rice blast disease is a serious agricultural problem mediated by infection-competent appressoria of the causal agent, *Magnaporthe grisea*. The non-reducing disaccharide trehalose is present in conidia of *M. grisea* and is mobilised during appressorium formation. The first step in trehalose biosynthesis involves trehalose-6-phosphate synthase (Tps1), and deletion of *TPS1* results in loss of pathogenicity. In addition, we have shown that the Tps1 protein is necessary for the regulation of several diverse cellular processes such as NADPH production and the ability to utilise nitrate as sole nitrogen source. This regulation occurs at both the transcriptional and post-translational level via glucose-6-phosphate dehydrogenase activity (resulting in reduced NADPH production), and misregulation of *NMR1*, an inhibitor of nitrogen metabolism, in *Dtps1* strains. Moreover, although independent of trehalose biosynthesis, the regulatory roles of Tps1 are dependent on efficient binding of glucose-6-phosphate (G6P) to the protein. Therefore, Tps1 integrates carbon and nitrogen signaling via G6P sensing. Here we show that a family of *NMR*-like genes - *NMR1, NMR2 and NMR3*- are present in the *M. grisea* genome. All three *NMR* genes act as extragenic suppressors of *Dtps1*, restoring the ability of *Dtps1* strains to cause rice blast disease. We propose that Tps1, in response to G6P sensing, regulates virulence-associated gene expression, conidiogenesis and nitrogen metabolism via control of NADPH levels and the NMR protein complex. Identification of genes regulated by the NMR complex, and a proposed model for its mechanism of action, will be presented.

Nitrogen controls invasive growth and plant pathogenicity in *Fusarium oxysporum* via the Ser/Thr kinase TOR and the bZIP transcription factor MeaB.

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During infection, fungal pathogens activate virulence mechanisms such as host adhesion, penetration and invasive growth. In the vascular wilt fungus *Fusarium oxysporum*, a mitogen-activated protein kinase (MAPK), Fmk1, was shown to be required for efficient root adhesion, penetration of cellophane sheets and plant infection. Here we studied the role of nitrogen regulation in the control of these virulence functions. Root adhesion and cellophane penetration were strongly impaired in presence of the preferential nitrogen source ammonium. By contrast, *F. oxysporum* mutants lacking MeaB, a bZIP transcription factor that mediates nitrogen metabolite repression in *Aspergillus*, still performed root adhesion and cellophane penetration in the presence of ammonium. Deletion of *meaB* did not restore root adhesion and cellophane penetration in a *fmk1* mutant, suggesting that MeaB and Fmk1 regulate these virulence functions through separate pathways. Interestingly, tomato plants supplied with ammonium, rather than nitrate, showed a significant reduction of vascular wilt symptoms when infected by the wild type strain, but not the *meaB* mutant. Rapamycin, a specific inhibitor of the conserved Ser/Thr kinase TOR, restored adhesion and cellophane penetration of *F. oxysporum* in the presence of ammonium. Two other plant pathogens, the rice blast fungus *Magnaporthe grisea* and the wheat head blight fungus *F. graminearum*, also showed repression of cellophane penetration by ammonium. Our results suggest that a conserved nitrogen response pathway operating via TOR and MeaB controls a subset of virulence functions in plant pathogenic fungi. (Poster # 459)

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Metabolomics and Proteomics: Peter Solomon and Kim Hammond Kosack

DON mycotoxin biosynthesis by Fusarium species, a metabolomics perspective.

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Many Fusarium species are fungal plant pathogens causing disease on both cereal and non-cereal hosts. Infection of the wheat ear typically results in bleaching and a subsequent reduction in grain yield. A larger proportion of the harvested grain may be spoiled by trichothecene mycotoxins such as deoxynivalenol (DON). Much progress has been made in the elucidation of genes required for trichothecene production, most of which are clustered at a single locus in the Fusarium genome. This project seeks to describe the metabolic characteristics associated with DON biosynthesis. As part of a new UK metabolomics initiative, we examined a range of well characterised wild-type Fusarium laboratory strains and single-gene deletion mutants under controlled conditions in vitro. A 'triple-fingerprint' of analytical techniques were employed to analyse the metabolome, composed of 1H-NMR and electrospray mass-spectroscopy (+/- ESI-MS). Principal components analyses of spectra were able to resolve *Fusarium graminearum, F. culmorum, F. pseudograminearum* and *F. venenatum* isolates after growth in liquid medium. In addition, several single-gene deletion strains that are reduced in pathogenicity exhibited large shifts in primary metabolism. Future work will attempt to find correlations between observed metabolic trends and DON biosynthesis.

Comparative proteomic analysis of infection-related development in the rice blast fungus *Magnaporthe oryzae*

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Rice blast disease is caused by the heterothallic ascomycete fungus *Magnaporthe oryzae* and is one of the most severe diseases of cultivated rice throughout the world. The *PMK1* (Pathogenicity Mitogen-activated protein Kinase) gene in *M. oryzae* has been shown to play diverse roles during pathogenesis-related development. *PMK1* regulates appressorium formation and infectious hyphal growth. *PMK1* is functionally related to *Saccharomyces cerevisiae FUS3*/*KSS1* MAPK genes which regulate the *STE12* transcription factor. The *STE12* homologue in *M. grisea*, *MST12*, has also been identified and is essential for appressorium mediated penetration and infectious growth. These observations imply that Pmk1 regulates a diverse set of targets important in both the initiation of appressorium development and the subsequent stages of invasive growth. The interplay between these two signaling proteins is currently being studied using a comparative proteomics approach in which we have defined the major proteomic changes associated with appressorium development in a wild-type rice pathogenic strain of the fungus and compared these with protein extracts from isogenic *Dpmk1* and *Dmst12* mutants. We have identified more than 600 differentially expressed proteins during conidium germination and appressorium formation by LC-MS/MS. The proteins have helped to define the morphogenetic processes regulated by the Pmk1 MAPK pathway and we have assessed the roles of a sub-set of proteins in appressorium morphogenesis using target gene replacement. We will present the results of our comparative proteomics analysis and the putative roles of the identified proteins in initiation of rice blast disease.

Proteome maps of total cell, mitochondrial and secreted proteins of *Aspergillus fumigatus*.

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Aspergillus fumigatus is a ubiquitously distributed saprophytic mould. Upon inhalation, *A. fumigatus* spores germinate, undergo hyphal growth and spread in the lungs causing deadly invasive aspergillosis in immunocompromised patients. In comparison to other fungi, *A. fumigatus* possesses obviously higher stress tolerance and better mechanisms to adapt to the host environment, since this *Aspergillus* species is the prominent cause for such severe lung infections. As a basis for comparative proteomic studies, we established a reference proteome map. Using MALDI-TOF-MS/MS we identified 392 protein spots representing 344 proteins separated on 2D-gels. Proteins involved in primary metabolism, protein synthesis, cell cycle regulation, DNA processing and transcription, cellular organisation and stress response were most abundant. Since mitochondria are involved in many essential processes such as energy metabolism, cellular differentiation and cell death, we established a protocol for the isolation of mitochondria of *A. fumigatus* mycelium and constructed a 2-D reference-gel for this organelle as well. 136 proteins represented by 196 spots have been identified so far. To complete the analysis of the mycelial proteome, we analysed the secreted proteins (secretome) under in vitro conditions. In many cases, proteins secreted by pathogens play an important role for their virulence. For *A. fumigatus* only little information about secreted proteins and their contribution to virulence is available. About 80 different proteins, represented by 272 spots, were detected of which approximately 45% were predicted as secreted proteins by bioinformatic analysis. Proteases and proteins involved in cell wall metabolism were most abundant. (Poster # 15)

Peptide-assisted annotation of the *Melampsora larici-populina* genome

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Annotation of protein-coding genes is a key goal of genome sequencing projects. In spite of advances in gene finder programs, computational identification of complete gene models in eukaryote genomes remains a challenging task. Proteomic data and bioinformatics were used to refine the annotation of the poplar leaf rust fungus *M. larici-populina*. Total proteins were extracted from urediniospores, fractionated by 1D SDS-PAGE, trypsin-digested and analysed by mass spectrometry. A corpus of 876 000 tandem mass spectra was searched against two protein databases: a database containing the protein sequences from the ab initio predicted gene catalog, and a second protein database created by a six-frame translation of the genome. Identified peptides validated the current annotation of 1659 genes, which represent about 10% of the predicted genes in *M. larici-populina*. Peptides only found on the six-frame translation database suggested modification to the current annotation for 99 genes and identified 73 novel genes that were not part of the *M. larici-populina* protein-coding gene catalog. Our results highlight the benefit of integrating proteomic data to genome sequencing project. (Poster # 605)

A dual 'omics approach to characterise asexual sporulation and toxin production in *Stagonospora nodorum*.

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Stagonospora nodorum is a major fungal pathogen of wheat. It has been previously shown by this laboratory that mutants defective in heterotrimeric G protein and MAPK signalling were affected in pathogenicity and showed developmental defects. In this study, we sought to gain a better understanding of signal transduction and phytopathogenicity in *S. nodorum* through the identification and characterisation of signalling target proteins. A comparative analysis of the *S. nodorum* wildtype and signalling mutants with 2-DE proteomics has led to the identification of a putative short-chain dehydrogenase (Sch1) that is subjected to positive regulation by both signalling pathways. Real-time PCR and transcriptional GFP fusion expression analyses have revealed that the *Sch1* is strongly expressed during asexual sporulation. Mutants lacking *Sch1* were altered in vegetative growth and showed a strong reduction in asexual sporulation. Detailed histological studies of the *sch1* mutants revealed a role for gene in facilitating the development of the fertile sub-parietal layer of asexual pycnidia and consequent sporulation. In addition, comprehensive non-targeted metabolomic analyses of the *sch1* mutants identified the strong accumulation of a metabolite positively identified as the mycotoxin alternariol. This is a first report that confirms the presence of a post-harvest mycotoxin in *S. nodorum*.

Quantitative proteomic analysis of an obligate bean rust fungus and its host

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Uromyces appendiculatus is a rust fungus that causes disease on the economically important dry bean plant *Phaseolus vulgaris*. The genome for *U. appendiculatus* has not been sequenced and the organism is genetically intractable. These barriers have made it difficult to use common molecular techniques to understand its biology. As a result, we have employed high-throughput liquid chromatography tandem mass spectrometry to quantitatively compare the proteomes of inactive asexual uredospores and germinating spores. Generally, the measured changes in accumulation for hundreds of proteins are indicative of metabolic transition from dormancy. These results are supported by cytological and developmental models of germling growth. In addition, we have statistically analyzed more than 3,000 proteins from infected bean leaves and have distinguished resistance from susceptibility at a proteomic level. Several of the plant proteomic responses appear to favor the pathogen during the course of infection. These results provide a basic foundation for rust fungal biology.

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The genome of *Blumeria graminis*: massive size expansion in an obligate biotroph
The BluGen consortium, Pietro Spanu et al, www.BluGen.org

We have sequenced the genome of *Blumeria graminis f. sp. hordei* (DH14). About 10x coverage of the genome was achieved using Sanger, 454 and Illumina sequencing. The genome is estimated to be around 120Mb, which is about 3-4 times larger than related Ascomycetes. The increase is due to an abnormal expansion of transposable elements (>70% of the genome), most of which are novel and uncharacterised (See Amselem *et al.*, this meeting) whilst the size non-repetitive gene space is congruent with similar fungi. The repeats are highly interspersed and are the result of ongoing active retro-transposition. We are currently annotating the genome using *ab initio* and similarity (EST mining and proteogenomics) based gene prediction pipeline. Conventional cDNA sequencing of 6 different developmental stages and high- throughput sequencing of 5' cDNA ends from sporulating epiphytic hyphae has lead to the definition of over 7700 unique genes (out of predicted total of ~11000) and to the discovery of high heterogeneity at the start of transcription, including the frequent presence of non-template bases. Proteogenomics of conidia, epiphytic hyphae and infected barley epidermis identify experimentally over 600 proteins. This reveals changes in the *Blumeria* proteome during development and disease, including a large complement of secreted proteins produced specifically by haustoria. It is expected that completion of the annotation including manual curation of the data will provide unique insights in the evolution, development and biology of powdery mildews and obligate biotrophy. (Poster 71)

High resolution analysis of fungal secreted proteins.
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Fungal-plant interactions are widespread. For instance, most tree species develop symbiotic relationships with fungi to enhance nutrient absorption, whilst many crop diseases that result in massive yield losses are caused by fungi. Little is known about proteins involved in these associations, but secreted proteins (secretomes) are predicted to play a key role. We present the first secretome analysis of fungi interacting with plants, the tree symbiont *Laccaria bicolor* and the rapeseed pathogen *Leptosphaeria maculans*. In this analysis, gel-based and gel-free proteomic approaches combined to mass spectrometry (MS) are exploited. Fungi grown in liquid media, with or without plant extracts were analysed. Fungal secreted proteins were recovered from medium filtrates. Several extraction methods were tested in combination with three proteomic techniques: two-dimensional electrophoresis (2-DE) followed by MS analyses, isoelectric focusing (IEF) followed by MS analyses (IPG shotgun) and one-dimensional electrophoresis followed by MS analyses (SDS-PAGE shotgun). The best 2-D gel resolution was achieved through an initial pre-fractionation using liquid-phase IEF followed by classic IPG-IEF/SDS-PAGE with narrow pH ranges. Hundreds of 2-D spots were thus resolved. In both species most of the secreted proteins had unknown functions. Of the ones with matches, most of them were to enzymes involved in cell wall modification or protein metabolism. This study paves the way for *in vivo* experiments and characterization of the fungal extracellular proteins involved in plant- fungus interaction. (Poster #469)

Associations with microbes and insects: Alfredo Herrera-Estrella and Pieter van West

Crazy ants: the fine detailed manipulation and exploitation of ants by the fungus *Ophiocordyceps (=Cordyceps) unilateralis*
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The fungus *Ophiocordyceps (=Cordyceps) unilateralis* manipulates ant behavior. In Thai rainforests infected Carpenter ants (*Camponotus leonardi*) leave their colony in the high canopy and descend 20m to the forest floor. Infected ants find and bite onto the underside of leaves. They preferentially choose leaves within a narrow band 25cm off the ground and on the North side of the plant. The timing of biting is highly synchronized around noon. Ants die a few hours after biting the leaf. Dead ants remain attached to the leaf for a year and in some cases 18 months. Unlike other entomopathogens *O. unilateralis* has evolved a sophisticated storage strategy and division of labor that facilitates such a long term strategy. The mechanism of behavioral change is targeted destruction of a number of mandible muscle organelles: myofibrils, mitochondria and sarcoplasmic reticula. Ants bite leaves because the fungus induces lockjaw. The sophisticated adaptations by this fungus to manipulate and exploit its living and dead host challenges our current model of fungal- insect parasite systems. We are developing a functional genomic approach, that includes de novo genome sequencing, to explore the proximate mechanisms of behavioral change in greater detail. (Poster # 508)

Signal transduction, secondary metabolism and *Trichoderma*-fungal interactions
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Trichoderma spp. directly affect many plant pathogenic fungi through mycoparasitism (of hyphae as well as the resting structures) and antibiosis, resulting in reduced crop loss, when applied as a biofungicide. Our earlier results demonstrated that the pathogenicity MAP kinase homologue TmkA regulates the parasitism of the sclerotia of *Sclerotium rolfsii*, and biocontrol efficacy against this pathogen. The deletion of the cell-wall integrity MAP kinase TmkB also affects the parasitism of this pathogen, and several other phenotypes overlap with *tmkA*, such as light-independent conidiation. Using differential screening by suppression subtractive hybridization (SSH), we are testing the hypothesis that pH detection via PacC provides a signal in the mycoparasitic interaction. Also by SSH, we identified a small, cysteine-rich secreted protein Mrsp1 that is down-regulated by TmkA. Interestingly, TmkA also downregulates GliT, a gene involved in the gliotoxin formation in *Aspergillus fumigatus*. Deletion of three G-protein alpha subunits (TgaA, TgaB, TgaC) individually has no significant effect on the growth and conidiation of *T. virens*, although the deletion of adenylate cyclase (Tac1) dramatically reduces the growth rate. This is in contrast with *T. atroviride*, where deletion of either Tga1or Tga2 affects growth and conidiation. The deletion of Tac1 also reduces the production of the secondary metabolites viridin/viridiol. To elucidate the role of secondary metabolites in interactions of *T. virens* with plant pathogens, we are systemically deleting the non-ribosomal peptide synthetases (NRPS), including GliP (gliotoxin) and siderophore-forming enzymes. The role of these NRPSs in *Trichoderma*-plant pathogen interactions and their regulation will be discussed.

Upregulated fungal genes in the early developmental stages of lichen symbiosis.
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One fifth of all known fungi are obligatory symbiotic partners with green algae, cyanobacteria, or both. An emergent property of this symbiosis is the formation of a lichen thallus. Although lichens are classic in illustrating mutualistic relationships, slow growth and genetic intractability have limited our progress in understanding the molecular basis to lichen development. Using the symbiosis between the fungus *Cladonia grayi* and the green alga *Asterochloris* sp., we have investigated differentially expressed genes in early thallus development. We used Suppression Subtractive Hybridization to find up-regulated genes in the early developmental stages between *C. grayi* and *Asterochloris* sp. in *in vitro* resynthesis. Among hundreds of identified gene fragments we chose candidate fungal genes to confirm differential gene expression using quantitative PCR. Our results parallel those seen in the mutualistic interactions between mycorrhizal fungi and their hosts: The development of both mycorrhizae and lichens appear to involve epistatic interactions between genes showing slight quantitative variation. The results of these experiments will be discussed and put into the context of putative roles in the lichens symbiosis and fungal symbiotrophy in general. (Poster # 581)

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Comparative studies of the secretome of fungus-growing ants.

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Leafcutter ants of the species *Acromyrmex echinatior* live in symbiosis with the fungus *Leucoagaricus gongylophorus*. The ants harvest fragments of leaves and carry them to the nest where they place the material on the fungal colony. The fungus secretes a wide array of proteins to degrade the leaves into nutrients that the ants can feed on. The focus of this study is to discover, characterize and compare the secreted proteins. In order to do so cDNA libraries are constructed from mRNA extracted from the fungus material. The most efficient technology to screen cDNA libraries selectively for secreted and membrane-bound proteins is the TAST (Transposon Assisted Signal Trapping) technology (Becker et al. 2004,Microbiol Methods 57, 123-133). The TAST screening will give a list of full length gene sequences encoding secreted proteins. The main part of the secretome will consist of biomass degrading enzymes, but also antimicrobial proteins will probably be in the secretome. To date only a few enzymes from the *Leucoagaricus* secretome have been identified. We expect to discover novel proteins and to gain a better understanding of the biodegrading pathways of *Leucoagaricus*. Ultimately, this work may identify enzymes that can be used in biomass conversion processes. (Poster # 426)

Cellular responses of *Phytophthora infestans* to cyclic lipopeptide surfactants produced by *Pseudomonas* species

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Oomycete pathogens cause devastating diseases on plants and animals and their control heavily depends on agrochemicals. With increasing concerns about adverse effects of agrochemicals on food safety and environment the development of novel, environmentally friendly control strategies, preferably based on natural products, is demanded. Cyclic lipopeptides (CLPs) produced by *Pseudomonas* species were discovered as a new class of natural compounds with strong activity against oomycetes including the late blight pathogen *Phytophthora infestans*. The *Pseudomonas fluorescens* CLP massetolide A (MassA), has zoosporicidal activity, induces systemic resistance and reduces late blight in tomato. To gain further insight in the modes of action of CLPs, effects on mycelial growth, sporangia formation, and zoospore behavior were investigated, as well as the involvement of G-proteins in sensitivity of *P. infestans* to MassA. In addition to zoospore lysis, MassA disturbed other developmental stages in the life cycle of *P. infestans*. G alpha gain-of-function mutants were less sensitive to MassA suggesting involvement of G-protein signaling in the response of *P. infestans* to this CLP. In order to reveal primary targets of CLPs we also monitored genome wide changes in gene expression. A distinct set of genes appeared to be up- or down-regulated after exposure to MassA, including genes encoding membrane transporters, alkaline phosphatases and pirins. Further characterization of these genes is in progress. (Poster # 145)

A lectin-mediated defense of fungi against predators and parasites

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The galectins CGL1 and CGL2 as well as the homologous lectin CGL3 of the homobasidiomycete *Coprinopsis cinerea* are strongly induced during sexual development and highly enriched in the fruiting body. Neither ectopic expression during vegetative growth nor silencing of the respective genes had any obvious effect on fruiting, suggesting that these lectins are not involved in mushroom development. Instead we found a pronounced, carbohydrate-binding-dependent toxicity of the galectin CGL2 towards the nematode *Caenorhabditis elegans*, two different insect larvae (*Aedes aegypti, Drosophila melanogaster*) as well as two different types of amoebae (*Acanthamoeba castellanii, Dictyostelium discoideum*). We tested a panel of additional characterized fungal lectins for toxicity towards the above mentioned organisms by feeding them with E. coli cells expressing the respective proteins. In this type of assay, most of these lectins were toxic and some were very selective with regard to the target organisms. Glycan-array analysis, in combination with genetic studies in *C. elegans*, allowed us to determine the target glycans of toxic fungal lectins and to start to unravel the mechanism of lectin-mediated nematotoxicity. Furthermore, we set up laboratory cultures of the fungivore nematode *Aphelenchus avenae* in order to evaluate the physiological significance of nematotoxic lectins for the fungus in co-culture with this predator. Our data suggest that the presence of the predator is able to induce the expression of CGL2 in the vegetative mycelium of both, monokaryotic and dikaryotic *C.cinerea* strains. We propose that homobasidiomycetes and possibly other multicellular fungi have an inducible innate immune system that includes a lectin-mediated defense against predators and parasites. (Poster # 613)

Intracellular pathogensis of filamentous fungi by the biocontrol bacterium *Lysobacter enzymogenes*.

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The gram negative bacterium *Lysobacter enzymogenes* infects a wide range of lower eukaryotes including filamentous fungi via internalization. We have been studying the molecular basis of *L. enzymogenes* with the filamentous ascomycetes *Cryphonectria parasitica* and *Magnaporthe oryzae*. The genome sequence of *L. enzymogenes* strain C3 is 6.1 Mb with a GC content of 69.5%. Initial global transcription and proteomic analysis have identified candidate genes regulated during the host-pathogen interaction. Further, *L. enzymogenes* appears to have at least one copy of every bacterial secretion system characterized to date. Bacterial secretion systems are intricate translocation machines that are often associated with pathogenicity and virulence of plants and animals. Using directed mutagenesis, we have been investigating the roles of Type 3, Type 4, and now Type 6 secretion systems on pathogenesis. *L. enzymogenes* appears to have two T6SS which contain the hallmark proteins present in all T6SS. To assess the roles of the T6SS pathways on lower eukaryotes, single and double deletion mutants of strain C3 lacking the Hcp proteins in each T6SS pathway are currently being constructed and the effect of the mutants on fungal infection will be monitored microscopically. (Poster # 94)

Analysis of gene expression and antibiotic production in biocontrol by Trichoderma spp.

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Trichoderma species are mycoparasites of phytopathogenic fungi. Upon recognition of a host they form specialized structures, secrete a complex set of cell wall degrading enzymes, and produce antibiotics. Therefore, during the interaction with the host there are important changes in gene expression. We have analyzed changes in gene expression in the interaction with different hosts in search for host specific genes by direct counting of cDNA sequences. Our analysis suggests that there are, indeed, host specific responses. In addition, we have generated mutants in the cfwA gene of T. virens, which are affected in the production of non-ribosomal peptides and polyketides. This approach allowed us to identify novel antibiotics produced by T. virens. In order to determine the relevance of such secondary metabolites for plant protection we analyzed the behavior of the mutants both in vitro and in vivo. The mutants clearly overgrow the host but are incapable of killing it, even in vitro, and are affected in their capacity to promote growth of tomato seedlings. (Poster # 494)

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Dothideomycete comparative genomics: Steve Goodwin and Gert HJ Kema

Development of genomic resources and tools for *Cochliobolus sativus*

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Cochliobolus sativus is the causal agent of three economically important diseases of barley and wheat: spot blotch, common root rot, and black point. To clone and characterize virulence genes in *C. sativus* and develop an understanding of the genetic and molecular interactions with the two major hosts (barley and wheat), we developed various genomic resources and tools for the fungus. Fungal crosses were made between two isolates (ND90Pr and ND93-1) of *C. sativus* that exhibit a differential virulence pattern on barley and wheat and classical genetic analysis showed that the virulence on barley and wheat is controlled by a single gene locus, respectively. A molecular map of *C. sativus* was constructed using AFLP and RFLP markers and six DNA markers were identified to be associated with a virulence locus for barley. Pulse field gel electrophoresis combined with telomere analysis indicated that *C. sativus* has a genome size of 33 Mb. A Fosmid DNA library was constructed for each of the two parental isolates (ND90Pr and ND93-1) with a 16X genome coverage. A bacterial artificial chromosome (BAC) library was also constructed for the isolate ND90Pr and 7200 clones with an average insert size of 110 kb are available for gene screening. In addition, more than 8,000 cDNA clones were sequenced and 7,200 ESTs were generated. Protoplast- mediated transformation system has been developed for gene knock out and for gene function characterization. RNA-mediated gene silencing system based on a Gateway cloning was established and optimized using the green fluorescence protein (GFP) gene and the Tox A gene from the wheat tan spot pathogen, *Pyrenophora tritici-repentis*. With the development of these genomic resources and tools, we are cloning and characterizing genes of interests in *C. sativus*. (Poster # 111)

A systematic analysis of T-DNA insertion patterns in the genome of *Leptosphaeria maculans*

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Agrobacterium tumefaciens-mediated transformation (ATMT) has been used for the transformation of more than seventy fungal species. ATMT offers numerous advantages over more conventional transformation methods and has led to the identification of genes involved in pathogenicity, pigmentation, conidial morphology and germination. However, data on the chromosomal distribution of the T-DNAs are scarce especially in fungi because, in most studies, only a few flanking sequences were rescued. As part of a large-scale insertional mutagenesis project aiming at the discovery of pathogenicity factors in the Dothideomycete fungus *Leptosphaeria maculans*, a collection of more than 5000 transformants has been generated and a set of 250 flanking sequences was obtained by thermal asymmetric interlaced-PCR and PCR-walking. The *L. maculans* whole genome sequence showed a very unusual genome structure for ascomycete fungi: the alternation of large GC-rich and AT-rich regions similar to isochores of higher eukaryotes. Genome wide plotting of T-DNA tags revealed a bias of insertions in favour of GC-rich isochores of larger chromosomes. Within the latter, integration events were significantly higher in intergenic sequences of gene-rich regions (60%). Besides, 81% of T-DNA's insertions in predicted genes occurred in exons. Further analysis of genes and genomic sequences surrounding the insertion sites will be presented. (Poster # 64)

Morphogenetic mutants of *Phoma medicaginis*

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Phoma medicaginis is the causal agent of spring black stem and leaf spot on alfalfa (*Medicago sativa*) and related medics, including the model legume (*M. truncatula*). This necrotrophic pleosporalean fungus produces melanized pycnidia, which exude conidia, on the necrotized tissues of its host leaves and stems. Conidia are disseminated by rain splash and act as the principle inoculum. The genome of *P. medicaginis* has not been sequenced; therefore we used a forward genetics approach to study pycnidial development and pathogenicity. Using *Agrobacterium* mediated transformation, over one-thousand transformants have been generated. From these, six morphogenic mutants with single T-DNA tags and altered melanization patterns, or aberrant or absent pycnidia, were selected. An adaptor ligation PCR method was used to obtain genomic sequence flanking T-DNAs and identified three types of T- DNA insertions. In the first type, the T-DNA insertions disrupted the coding regions of a polyketide synthase (*pks*) and a poly-A polymerase (*cid13*). In the second type, the T-DNAs inserted in the promoter region or 3'-UTR of a cyclin-like protein and an unknown hypothetical protein conserved in eukaryotes, respectively. In the final type, the T-DNAs appeared to have integrated into intergenic regions. The transcription of tagged genes will be verified and open reading frames will be deleted by gene replacement to functionally regenerate mutant phenotypes. Efficient T-DNA tagging, a model host plant, and numerous genome sequences of related dothideomycete fungi make *P. medicaginis* a promising model plant pathogen. (Poster # 596)

Comparative genomics tools for analysis of six Dothideomycete genomes

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Comparative genomics is a powerful tool for genome annotation and analysis. We recently initiated analysis of six of the Dothideomycete genomes that are publicly available: *Mycosphaerella graminicola, Mycosphaerella fijiensis and Cochliobolus heterostropus C5* all sequenced and annotated at the DOE Joint Genome Institute as well as *Alternaria brassicicola* sequenced at the Washington University School of Medicine, Genome Sequencing Center and annotated by the Christopher Lawrence Lab at Virginia Bioinformatics Institute, Virginia Tech, *Pyrenophora tritici-repentis and Stagonospora nodorum*, both sequenced and annotated by the Broad Institute, MIT. We placed these genomes into a comparative framework equipped with tools to facilitate genome analysis: VISTA genome conservation curves linking genome browsers, gene cluster browser and protein links to homologs in all six genomes, side-by-side comparisons of KEGG pathways and KOG functions, and synteny viewer. JGI community annotation brought biologists behind each of these genomes together to explore common mechanisms of plant pathogenicity and host interactions. (Poster # 22)

Outstanding: the dispensable chromosomes of *Mycosphaerella graminicola*

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Analysis of two genetic linkage maps of the wheat pathogen *Mycosphaerella graminicola* identified dispensable chromosomes that were present in both parents but absent in 15-20 % of the progeny. These Copy number Polymorphisms (CNPs) were confirmed with a Comparative Genomic Hybridization whole-genome array based on the finished genome of *M. graminicola* (http://genome.jgi-psf.org). Chromosomes 14- 21 were frequently absent among isolates, without visible effect on viability or virulence, whereas chromosomes 1-13 were invariably present. Genetic analyses showed that CNPs arises during meiosis, usually from nondisjunction at anaphase II. Overall, *M. graminicola* has the highest number of dispensable chromosomes reported. Varying from 0.41 to 0.77 Mbp, they comprise 38% of the chromosome number and 11.6% of the genome. The dispensable chromosomes are smaller and have significantly lower gene densities. Most of their genes are duplicated on the essential chromosomes and show a different codon usage. Dispensable chromosomes also contained a higher density of transposons, pseudogenes, and unclassified genes, which could encode novel proteins. Moreover, the dispensable chromosomes show extremely low synteny with other Dothideomycete genomes. We hypothesize that the dispensable chromosomes of *M. graminicola* are adaptive in some yet unknown way. (Poster # 102)

Absence of cytosine methylation in *Mycosphaerella graminicola* correlates with Repeat Induced Point mutation signatures in its *Dim-2* homologue.

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A *de novo* search for repetitive elements in the genome sequence of wheat pathogen *Mycosphaerella graminicola* identified a family of repeats containing a DNA methyltransferase gene, which is a homologue of the *Neurospora crassa Dim-2* gene. A total of 31 *MgDim-2* sequences were identified, all of which carried signatures of Repeat Induced Point mutations (RIP). All *MgDim-2* copies were sub-telomeric in location, except for one copy on chromosome 6. Synteny of this non-telomeric *MgDim-2* region to a closely related fungus, *M. fijiensis*, implied that the copy on chromosome 6 was the original sequence that was amplified. Amplification of the *MgDim-2* gene in 14 other *M. graminicola* isolates from various geographical regions was substantiated by Southern analysis. However, an *in silico* search for *MgDim-2* homologues identified a single, unRIPed copy in eight ascomycete genomes, suggesting that the amplification event might be specific to *M. graminicola*. A genome-wide assay by ESI-MS/MS revealed that cytosine methylation was present in *M. fijiensis* but absent in *M. graminicola*, as expected if the *MgDim-2* gene is inactivated. Our results indicate that amplification of the single-copy *MgDim-2* gene made it susceptible to RIP, which might be responsible for the complete lack of cytosine methylation in *M. graminicola*. (Poster # 43)

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Homologues of the *Cladosporium fulvum* effector proteins are present in Mycosphaerella species

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Cladosporium fulvum is a non-obligate biotrophic fungal pathogen that causes leaf mould of tomato. Until now, ten effector proteins have been identified from *C. fulvum* that historically have been divided into avirulence (Avrs: Avr2, Avr4, Avr4E and Avr9) and extracellular proteins (Ecps: Ecp1, Ecp2, Ecp4, Ecp5, Ecp6 and Ecp7), and whose recognition in tomato is mediated by their cognate Cf (for *C. fulvum*) resistance proteins. Although demonstrated for only some of them, all Avrs and Ecps are assumed to be virulence factors. Avr2 is an inhibitor of apoplastic plant cysteine proteases, while Avr4 encodes a chitin-binding protein that protects chitin present in the cell walls of the fungus against the deleterious effects of plant chitinanes during infection. Ecp6 contains carbohydrate/chitin-binding LysM domains that putatively bind chitin fragments released from fungal cell walls during infection and prevents them from inducing basal defense responses during infection. Despite the fact that the interaction between *C. fulvum* and tomato is a model to study gene-for-gene based interactions, so far no homologues of the *C. fulvum* effector proteins have been identified in other fungal species. However, by mining of sequenced fungal genomes for homologous nucleotide sequences and protein motifs, we have now identified for the first time homologues of the *C. fulvum* Avr4, Ecp2 and Ecp6 effectors in other fungal pathogens, including *Mycosphaerella fijiensis*, *Mycosphaerella graminicola* and *Cercospora nicotianae*. Recently we have tested whether the three *M. fijiensis* homologues are truly functional homologues of the three *C. fulvum* effectors. We will report on the exciting results that have been obtained from these studies. (Poster # 539)

Deciphering the genetic basis of speciation of a fungal plant pathogen through comparative genomics

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We compare the genome sequence of the wheat pathogen *Mycosphaerella graminicola* to the genome of a closely related pathogen (here named S1) infecting wild grasses to decipher patterns of genome evolution during speciation. Previous coalescence studies show that these two pathogens diverged 11.000 years ago in the Fertile Crescent coinciding with wheat domestication. We sequenced the genome of S1 using Solexa sequencing to an average coverage of 40X. Paired end reads were assembled using both a de novo assembly approach and by reference assembly to the already sequenced genome of *M. graminicola*. The assembled genome of S1 covers ~85% of the non repetitive fraction of *M. graminicola* nuclear chromosomes. Several structural rearrangements have occurred affecting mainly the seven dispensable chromosomes of *M. graminicola*. ~7Mb bases of sequence in S1 could not be aligned to the *M. graminicola* genome representing DNA that has either been lost from *M. graminicola* or inserted in S1 after their divergence. The 43kb mitochondrial sequence of *M. graminicola* was used as reference for assembly of the S1 mitochondrial genome. We obtained a coverage of ~740X providing us with an opportunity to detect and analyze mutational derivates within the sequenced individual. Conflicting sites within the S1 mitochondrion supports previous findings of heteroplasmy in *M. graminicola*. (Poster # 389)

Speciation and evolution: Tatiana Giraud and Jan Schirawski

Mating-type chromosome evolution in the filamentous ascomycete *Neurospora tetrasperma*
Hanna Johannesson, Audrius Menkis and David Jacobson

We combined gene divergence data, classical genetics and phylogenetics to study the evolution of the mating-type chromosome in the filamentous ascomycete *Neurospora tetrasperma*. In this species, a large non-recombining region of the mating-type chromosome is associated with a unique fungal life cycle (pseudohomothallism) where self-fertility is enforced by maintenance of a constant state of heterokaryosis. Sequence divergence between alleles of 35 genes from the two single mating-type component strains (i.e. the homokaryotic *mat A* or *mat a*-strains), derived from one *N. tetrasperma* heterokaryon (*mat A + mat a*), was analyzed. By this approach we were able to identify the boundaries and size of the non-recombining region, and reveal insight into the history of recombination cessation. The non-recombining region covers almost 7 Mbp, over 75% of the chromosome. Furthermore, we found indications that the evolution of the mating-type chromosome in this lineage involved two successive events: two "evolutionary strata", highlighting shared features between the sex chromosomes found in the animal and plant kingdoms and the fungal mating-type chromosome, despite fungi having no separate sexes. Finally, in spite of an early origin of the first "evolutionary stratum", genealogies of 26 genes on the *mat*- chromosome from strains belonging to 9 reproductively isolated *N. tetrasperma* lineages indicate independent initiations and history of suppressed recombination in different phylogenetic lineages of the morphospecies.

Evidence that the Vancouver Island Cryptococcus gattii outbreak has expanded into the United States Pacific NW
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Cryptococcus neoformans and *Cryptococcus gattii* are common fungal mammalian pathogens. *C. neoformans* is more prevalent, associated with pigeons in nature and a frequent cause of meningitis in immunocompromised patients, whereas *C. gattii* is geographically restricted to tropical and subtropical regions, associated with trees and usually infects immunocompetent individuals. Since 1999, an outbreak of *C. gattii* on Vancouver Island, British Columbia, has become endemic, caused numerous human and veterinary infections, and spread to mainland British Columbia. The outbreak isolates of C. gattii were characterized as molecular type VGIIa/major or VGIIb/minor. Beginning in 2006, human and veterinary cases have emerged in Washington State and Oregon. Using high-resolution multilocus sequence typing at a minimum of eight unlinked loci, we determined that most of these strains were VGIIa/major or VGIIb/minor, which provides direct evidence for the emergent spread of *C. gattii* from Vancouver Island to the Pacific Northwest of the United States. In addition, five isolates unique to Oregon and related to the VGIIa/major genotype form a novel cluster, which we have termed VGIIc. In addition, highly variable non-coding regions are under examination to further detect genomic differences among the VGIIa/major outbreak isolates. Continued analysis of veterinary, human, and environmental isolates from the region is ongoing, and the *C. gattii* Working Group of the Pacific Northwest has been established as a multidisciplinary effort to study the emergence. This unusual outbreak in a temperate climate raises concern about further expansion in the region and illustrates how microbial pathogens emerge in novel geographic locales. (Poster #396)

Genetic variability of mating genes and virulence factors of phytopathogenic fungi
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Ustilago maydis, the causal agent of corn smut disease, is a well established model organism to investigate highly specific pathogen-host interactions. In such an intimate coexistance, the emergence of new species depends on parameters related to virulence and resistance. In consequence, evolutionary studies of genes involved in those interactions are essential for the understanding of speciation processes. Smuts like *U. maydis* are characterized by a dimorphic life cycle with a haploid yeast and a dikaryotic parasitic stage linked by mating. After penetration the fungus proliferates within the host and finally diploid teliospores are produced. During this biotrophic phase many genes are crucial for the establishment of the interaction. In 2006, Kaemper and coworkers identified 12 gene clusters of secreted proteins with decisive functions in the infection process. Ongoing functional genetic approaches focus on those „biotrophy islands" unveiling potential determinants of host specificity and virulence. Due to the direct interaction of parasites and hosts, speciation is linked to events of cospeciation, host shifts or host jumps. Therefore, we hypothesize that virulence factors are key players in speciation processes of the Ustilaginaceae. To test this, we performed a comparative population genetic approach investigating mutation rates of mating genes, potential virulence factors and housekeeping genes. Interestingly, mating genes show a similiar conservation than housekeeping genes indicating a low evolutionary rate. In contrast, virulence factors are highly variable proposing balancing selection on „biotrophy islands". Based on our dataset, we will discuss the contribution of different genes to speciation processes. (Poster # 418)

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Speciation and recombination in the globally distributed penicillin producing fungus *Penicillium chrysogenum*.
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Penicillium species generate numerous spores that are highly effective dispersal agents and often among the most abundant viable organisms in any environment, from deeply buried ice to the mesosphere. Nevertheless, most species display strikingly heterogeneous distributions, and multilocus sequencing efforts often reveal underlying genetic diversity that is further structured by environmental variables such as geographic distance or habitat. We used microsatellite markers and multilocus DNA sequencing of a historical and contemporary global collection of *P. chrysogenum* isolates was used to address four questions: 1) Does *Penicillium* section Chrysogena including *P. chrysogenum* contain "cryptic species" 2) Does *P. chrysogenum* undergo parasexual mitotic recombination or sexual meiotic recombination? 3) At what spatial scale does population genetic substructure exist? 4) Is the recombination rate and scale of population substructure conserved among extremely closely related or incipient species? We used a two tiered approach to delimit species. First, we used microsatellite allele frequency to assign individuals to as many as six putative species. We then used a coalescent based test of those species limits with multilocus sequence data. We detected five "cryptic species". Analyses revealed that linkage disequilibrium among markers was high and recombination rate was low relative to microsatellite mutation rate. However, recombination did occur between markers on contiguous DNA regions, suggesting that perhaps recombination is more likely to be meiotic rather than mitotic. Although infrequent, identical genotypes were recovered from isolates on different continents. Despite intensive sampling at a small spatial scale in the UK and broad global sampling, no spatially correlated recombination was detected.

Speciation genes in fungi.
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Speciation genes are determinants of reproductive isolation - death or fitness deficit among progeny of interspecies hybrids. The recent report by Lee et al. (2008) of such hybrid sterility between two species of Saccharomyces, owing to incompatibility between a nuclear-encoded mitochondrial regulatory protein and its mitochondrial-encoded target gene, could be the first example in fungi. The caveat is that speciation may well have preceded emergence of the genic incompatibility. We seek the determinants of hybrid sterility or fitness loss under conditions of incipient speciation in two fungal models: S. cerevisiae and Neurospora crassa. Initial experiments based on in vitro evolution among populations of 12 experimental lineages provided two main results in both systems: (1) ecological speciation through adaptation to two selective environments evidenced by deficits in mitotic fitness and meiotic efficiency of hybrids compared to the pure, adapted lineages, and (2) negative epistasis (Muller-Dobzhansky model) evidenced by decrease in fitness in progeny of hybrids between lineages adapted to the two selection regimes as compared to hybrids between the progenitor and the adapted lineage. Whole genome resequencing/Solexa of four evolved yeast haploids has revealed SNPs accounting for most of the adaptation in ecological speciation. Results include the expected, that DM negative interaction may occur between alleles conveying adaptation to both selection regimes in a hybrid. Less expected is the negative interaction between alleles conveying adaptation to one selection regime in matings between parallel-evolved lineages. In populations evolved from an N. crassa/N. intermedia cross, between two species presumably fully diverged long ago, two genes impose a fitness deficit on hybrids between females and males evolved under different selection regimes. We conclude that reproductive isolation is easily evolved in experimental populations and that it is likely due to many kinds of gene, gene product, and system interactions.

Inferring the history of speciation from multilocus sequence and microsatellite data: the case of the *Microbotryum violaceum* species complex
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Microbotryum violaceum has a rich scientific history, and is a model system for the study of the ecology and evolution of host/pathogen interactions, fungal genetics and ecology. This basidiomycete fungus causes anther-smut disease on more than 100 Caryophyllaceae, but constitutes a complex of sibling species restricted to different hosts. Previous studies have shown that barriers such as ecological isolation, F1 inviability and sterility, F2 sterility and selfing contribute to the maintenance of species in this system. However, the ease to produce hybrids in the lab for some close species pairs suggests that their boundaries may actually be semipermeable to gene flow. We used microsatellite markers to evaluate gene flow among natural populations of two sister species of *Microbotryum* (infecting *Silene latifolia* and *S. dioica*, respectively) that are known to produce viable and infectious F1 and F2 hybrids in the lab. Inter-specific hybrids (although few) were identified in zones where both host plants grow in sympatry, suggesting that gene flow may indeed occur between the two species, and that the geographic distribution of host plants may be an influential driver of this phenomenon. We therefore used microsatellite data to investigate the past distribution of each species in Europe and found that both exhibited the same East/West partitioning of genetic variation, consistent with a history of recolonization of Europe from the same glacial refugia. To determine whether genetic exchanges between the two species have been favored by their co-occurrence in the same regions during glaciations, we estimated the amount, direction and timing of gene flow between species from nucleotide polymorphism at multiple loci analyzed using inferential models based on coalescent genealogy samplers. The goal was to assess whether gene flow was a regular component of the speciation process (divergence-with-gene flow model of speciation) or whether genetic exchanges rather represent a byproduct of secondary contact during Pleistocene climate fluctuations.

Evidence for hybridization and introgression between *Coccidioides immitis* and *C. posadasii*

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Coccidioides is a soil-dwelling dimorphic fungus endemic to semi-arid areas of the Americas. This pathogen of humans and other mammals was chosen for comparative genomics at the species and population levels. Sequenced strains represent both species, with variation in biogeography, virulence, and isolate source. The nature of the species and population boundaries are of particular interest because of our recently discovered evidence of hybridization and introgression between species. Evidence for gene flow across the two species was detected by reciprocal blastn in all pairwise combinations for nonredundant 1kb fragments from four *C. immitis* and three *C. posadasii* genomes. Our analysis identified regions from each genome that had a closer match to the opposite species than its own. For most of the *C. posadasii* strains, there were few regions that had a closer match to *C. immitis*. However, there was one region within 3 of the 4 *C. immitis* strains that matched *C. posadasii*. This region contains a common border and a variable border. To assess the frequency of this *C. posadasii*-type region in *C. immitis*, a larger collection of isolates was screened. Approximately 30% of the *C. immitis* isolates contain this 70-120kb region, and the majority of those are from the southern California and Mexico populations. Our results suggest that hybridization has occurred between species, and at least one fragment appears to have introgressed in *C. immitis.* Additional Solexa sequencing confirms the observation. (Poster # 105)

Host adaptation as mechanism for speciation? Comparative analysis of *Sporisorium reilianum* isolates with different host preference.

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Sporisorium reilianum is the causative agent of head smut of maize and sorghum. Haploid strains of different mating type fuse and form infectious dikaryotic hyphae that can penetrate young leaves. Although hyphae of *S. reilianum* can be found in all plant tissues, sporulation takes place exclusively in the inflorescence. We isolated haploid strains from spores of diseased maize and sorghum plants. Infection assays showed that maize isolates of *S. reilianum* (SRM) are infectious on maize but not on sorghum, while sorghum isolates of *S. reilianum* (SRS) are infectious on sorghum but not on maize. SRM and SRS strains are mating competent, and crosses lead to (a low rate of) spore formation on maize. Thus, the isolated SRM and SRS strains are varieties of the same species.

Crosses of SRM and SRS strains do not cause sorghum disease and succumb to tissue-specific host defense. This suggests the existence of factors in SRM strains that inhibit virulence on sorghum. Segregation analysis indicated involvement of many genes in host adaptation. Using comparative genomics of maize and barley smut fungi we identified three genes with a potential inhibitory function for virulence on sorghum and supportive function for virulence on maize.

Genome comparison of both varieties supported that SRM and SRS strains represent a species under evolution that develops into two distinct species because of adaptation to different hosts.

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Abstracts for Posters

Comparative and Functional Genomics

1. Pathogenicity Determinants of *Fusarium graminearum* on Wheat Ears.
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Using a novel bioinformatics approach, we have identified a micro-region of verified pathogenicity gene homologues in the important crop pathogen *Fusarium graminearum*. This region is now being analysed by a combination of bioinformatic and reverse genetics approaches to ascertain its role in pathogenicity of this species with the aim of locating novel virulence determinants.
Comparative genomics has been used to investigate conservation of the micro-region in other closely and less closely related species. Targeted deletion of single genes has allowed the determination of the role of micro-region genes in *F. graminearum* pathogenicity.
Deletion of the neutral trehalase gene *NTH1* appears to slow infection of wheat ears, while deletion of the *SNF1* protein kinase or *PKAR* cAMP-dependent protein kinase regulatory subunit inhibits pathogen spread in wheat. In addition, a conserved hypothetical gene in the micro-region has also been shown to be required for full pathogenicity and so represents a new type of virulence factor.
This micro-region appears to be distinctly different from the virulence-associated biosynthetic and secreted protein clusters identified so far in pathogenic fungi. Further investigation will reveal more about the evolution of this small genomic region.

2. Origin and inheritance of a host-specific gene among closely and distantly related Fusarium spp.
Nick Milani and Scott Kroken. University of Arizona, Tucson. nmilani@email.arizona.edu

Fungal pathogens of pea (Pisum sativum) in the anamorphic genus Fusarium harbor a virulence gene, pisatin demethylase (PDA) that detoxifies pistatin, allowing for colonization. Previously noted correlations for the hypothesis of horizontal gene transfer (HGT) among these fungi include the discontinuous distribution of this gene and the presence of PDA in a virulence-enhancing gene cluster, Additionally, in (Haemato)nectria haematococca, the PDA gene cluster is on a conditionally dispensable chromosome, whose genes have a GC- and codon bias compared to genes on typical chromosomes. To test the hypothesis of HGT, the gene genealogy of PDA was compared to the organismal phylogeny of Nectria haematococca, Fusarium oxysporum fsp. pisi, and Neocosmospora boniensis. The genealogy is concordant with the organismal phylogeny, and the sequence-level divergence of the PDA genes is consistent with other vertically inherited genes, both supporting the null hypothesis of vertical inheritance. In addition to inferring a vertical mode of inheritance and gene loss among Fusaria that are not pathogens of pea (and other legumes that may make pisatin), the gene genealogy suggests that PDA originated from a cytochrome P450 via duplication and divergence, having been co-opted from a P450 gene family with roles in the modification of endogenous metabolites, to activity on an exogenous metabolite. To account for the other lines of evidence that had been used to propose HGT of PDA genes, alternative explanations for those observed phenomena will be presented.

3. Exploiting new sequencing technologies to study fungal genome structure and variation
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We have applied new sequencing technologies that generate large amounts of inexpensive data to genome assembly and polymorphism discovery in fungi. In doing so, we have developed new methods to maximize the utility of these new data types.
ALLPATHS is a graph theory-based Illumina read assembly algorithm that delivers highly accurate and contiguous assemblies for genomes of up to 40Mb in size, e.g. Schizosaccharomyces pombe, Candida albicans and Neurospora crassa. For example, our ALLPATHS assembly of S. pombe yields contig and scaffold N50 sizes of 230kb and 1.3Mb respectively (base accuracy >Q50) and covers 98% of the reference. Further, using 454 data exclusively or in combination with Sanger data we generate quality assemblies and can capture large regions of the genome absent from Sanger libraries, e.g. Neurospora crassa. For polymorphism discovery, we have developed VAAL, an algorithm that uses Illumina data to identify differences between microbial genomes at high sensitivity and specificity. VAAL typically detects ~98% of differences (SNPs to large indels) between pairs of related strains of bacteria while calling no false positives and is sensitive enough to pinpoint a single drug resistance mutation between bacterial genomes e.g. Vibrio. We have also used VAAL to identify polymorphisms in strains of Schizosaccharomyces and are applying this methodology to larger fungal genomes.

4. Sequence and annotation improvements to the *C. albicans* genome assembly, using comparative genomics and high throughput sequence data
Michael F. Lin[1,2], Martha B. Arnaud[3], Gail Binkley[3], Maria C. Costanzo[3], Stuart R. Miyasato[3], Prachi Shah[3], Marek S. Skrzypek[3], Christina A. Cuomo[1], Manolis Kellis[1,2], and Gavin Sherlock[3]. [1]Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA [2]Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA [3]*Candida* Genome Database (CGD), Department of Genetics, Stanford University Medical School, Stanford, CA

The *C. albicans* genome sequence was published by the Stanford Genome Technology Center (SGTC) as a set of 266 haploid contigs (Assembly 19; Jones et al, 2004), which were subsequently assembled by the BRI-Canada into 16 supercontigs aligned on 8 chromosomes (Assembly 21; van het Hoog et al, 2007). To improve the ORF annotation, we compared the sequences of eight *Candida* genomes and identified potential new ORFs, candidate "Dubious ORFs," incorrectly annotated ORF boundaries, and possible sequencing errors (Butler et al., submitted), in addition to the suspected errors ("adjustments") previously identified by the Annotation Working Group. Each of these predictions has now been curated and included in CGD. Regions containing suspected sequence errors were re-evaluated using sequence trace data from two sources: the trace archive from the SGTC, and a set of new data that we generated using the 454 platform, with a focus on correcting sequence errors that affect open reading frames. In each case, trace alignments were manually curated, and we have removed all of the ORF "adjustments" and have either corrected the sequence, where the sequence trace data supported a change, or updated the ORF boundaries. The changes that were made to each ORF are described in detail on its Locus History page, and Chromosome History pages describe all of the updates to the chromosomal sequences and their annotations that have been made since the release of the Assembly 21 reference sequence. We thank the Sanger Institute for the *C. dubliniensis* and *C. parapsilosis* data. CGD curators welcome comments and suggestions by email, at candida-curator@genome.stanford.edu CGD is supported by grant RO1 DE15873 from the NIDCR at the NIH.

5. Alignment of a genetic linkage map to whole-genome sequence assemblies for the pathogenic basidiomycete *Heterobasidion annosum*
Mårten Lind, Åke Olson, Jan Stenlid. Dept. of Forest Mycology and Pathology, Box 7026, SE-750 07 Uppsala, Sweden.

We have previously reported an AFLP-based genetic linkage map for *Heterobasidion annosum*, casual agent of annosum root rot in conifer forests. The map was constructed from a sample of 358 markers derived from a sporulating hybrid between North American P- and S-type isolates. Using whole-genome sequence assemblies for the P-type parental isolate, we have aligned the linkage groups of the map to the genome. This was done using a software which identified putative restriction sites used in the AFLP-analysis and provided fragment lengths for these sites. Fragment sizes within five bp of the corresponding AFLP marker were considered as candidates. When candidates from the same linkage group were found on a scaffold and positioned in coherence with their relative distance in cM in the linkage map, they were considered to represent the whole-genome sequence location of the linkage group. We were able to find putative positions for 80% of the AFLP-markers from the P-parent in the mapping population and through this align 18 of the 19 larger groups to the sequence. Presently, the positions of the aligned linkage groups are being verified by microsatellite markers identified within the sequence spanning the linkage groups. If these microsatellites link to the markers of the group, the position will be considered to be verified.
A linkage map fully aligned to the sequence will be a very useful tool to identify the various QTLs located in this map, controlling traits such as pathogenicity, intersterility and various intraspecific interactions.

6. A novel transmembrane protein is required for oxidative stress homeostasis and virulence in plant and animal fungal pathogens.
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Regulation of reactive oxygen species appears to be very important for pathogenic fungi during cell differentiation and pathogenesis. In this report we demonstrate that *Alternaria brassicicola* ATM1, a novel transmembrane protein, is necessary for tolerance to oxidative stress and plant pathogenesis. *ATM1* encodes a predicted hybrid membrane protein containing a single adenylation, six putative transmembrane, and FAD and NAD(P)-binding domains, and shows high sequence similarity to proteins found only in filamentous fungi including an animal pathogen *Aspergillus fumigatus*. Localization and gene expression analyses indicated that *ATM1* is associated with fungal Woronin body, a specialized peroxisome, and strongly expressed during conidiation and initial invasive growth in planta. *A. brassicicola ATM1*- deficient mutants exhibited abnormal conidiogenesis, accelerated loss of cell integrity of aged conidia, hypersensitivity to oxidative stress, and excessive oxidative burst of its own during conidiation and plant infection compared with wild-type strain. Virulence assay on green cabbage plant showed dramatically reduced virulence of the *Delta-atm1* mutants. Analysis of one of the *ATM1* homologs, *AtmA* in *A. fumigatus* revealed that the *ATM1* is functionally conserved in both plant and animal pathogenic fungi. Collectively, these results suggest that *ATM1* is likely to be involved in maintaining oxidative stress homeostasis during conidiation and pathogenesis.

7. A micro-array analysis of conidial germination of *Aspergillus niger*.
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The germination process of conidia of *Aspergillus niger* was analysed by means of micro-array analysis. Gene expression was studied in freshly harvested conidia, throughout isotropic growth and subsequent polarization. To this end, a protocol was developed to isolate RNA from freshly harvested and dormant spores. RNA of more than 5000 genes (~35 %) was detected in freshly harvested spores suggesting a high variability of gene expression. The signals were generally low, suggesting that spores contain a relatively low level of mRNA's during this stage. The variability of RNA dropped during early germination (2 h) but this was accompanied by an increase in the average intensity of the signals. The number of genes that were expressed increased to ~34% when germ tubes had been formed.

8. **Kinesin genes and nuclear migration in the homobasidiomycete *Schizophyllum commune***
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In *S. commune* intracellular nuclear migration occurs when the nuclei quickly separate after division. Intercellular nuclear migration is associated with the mating of haploid strains with different B-mating-type complexes and it involves passage of fertilizing nuclei from one mate into and through the hyphae of the other. In all these cases microtubules are connected with nuclei suggesting that the microtubule associated motor molecules are involved in nuclear movements. We have cloned the genomic and cDNA sequences of three kinesin genes. Sckin1 amino acid sequence is highly homologous with the conventional kinesin known to be involved in vesicle transport in filamentous fungi. Sckin2 shows high identity to kinBimC implicated in mitotic spindle function. *Sckin3* is highly homologous with a kinesin also present in *Laccaria bicolor* and *Coprinopsis cinerea*. By screening the pre-released version of *S. commune* genome at least seven additional kinesin genes are found, with which the number of kinesin genes is comparable to that in the genome of *C. cinerea* and *L. bicolor*. The expression of the different kinesin genes is screened by qRT- PCR during the fungal life cycle with special attention to their relationship to the expression of B-mating-type genes in order to reveal the role of kinesins in intra- and intercellular nuclear migration.

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9. Whole genome transcript analysis to identify *veA*-dependent genes in *Aspergilllus flavus* associated with aflatoxin production and fungal morphogenesis.

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VeA has been shown to regulate development and secondary metabolism in a number of fungal species including *Aspergillus flavus* where inactivation of *veA* results in loss of aflatoxin (AF) and sclerotial production. We have performed an experiment using *A. flavus* whole genome microarrays to look at differential expression of genes between the wild-type (WT) and a mutant *veA* strain. Results identified a number of genes that demonstrated a significant difference in expression between strains and over time. Gene ontology classification of these genes identified a group involved in nitrogen metabolism, including the nitrate reductase (*niaD*) gene. Microarray analysis showed that *niaD* expression was significantly lower in the *veA* mutant compared to the WT. Based on these results an *A. flavus* strain harboring a WT or mutant copy of *niaD* were examined for AF production and conidial and sclerotial formation following growth for 5 days. Microscopic examination of sclerotia showed that the *niaD* mutant produced fewer and smaller sclerotia than the WT strain. Analysis of conidial formation and AF production showed that the *niaD* mutant produced about 10-fold more conidia than the WT but less AF. These results indicate that nitrogen metabolism plays a role in both AF production and development in *A. flavus* and that expression of some of the genes involved are *veA*-dependent.

10. An initial look at nutrient-driven changes in global gene expression in *Coprinus cinereus*

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The saprophytic basidiomycete *Coprinus cinereus* grows in dung and leaf litter. In this substrate, most available nitrogen is sequestered in protein. Using microarrays supplied by Claire Burns and Mimi Zolan, we examined differences in gene expression in exponentially-growing monokaryotic mycelia in response to protein nitrogen source. Three-day-old mycelia were transferred from minimal medium (containing glucose and L-asparagine) to soy protein as sole nitrogen source, or as sole nitrogen *and* carbon source, for 16 hr. Control mycelia were transferred to fresh minimal medium. Approximately 4000 genes were differentially expressed (about half up-regulated and half down-regulated) in each treatment, with about half of those being unique to the treatment. Of the 100 most highly up-regulated genes in each case, only one was common to both treatments. Genes whose products are associated with hydrolysis of polysaccharides and proteins were significantly up-regulated when soy protein was the sole nitrogen *and* carbon source; genes involved in mating pheromone reception were significantly down-regulated. Regardless of carbon source, transfer to soy protein up-regulates about 25 % of the 301 peptidase genes. Thirty-eight up-regulated genes, encoding members of the five major mechanistic classes of peptidases, are common to both treatments. However, ten of the most highly up-regulated peptidase genes when soy protein is the sole N *and* C source are not up-regulated after transfer to soy plus glucose. These results suggest there may be a distinct mechanism for protease gene regulation that is responsive to carbon starvation.

11. Comparative analysis of transposable elements in several fungal genomes

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Transposable Elements (TEs) are repeated sequences whose abundance is usually correlated with genome size. TEs shapes genomes and are a source of genetic variations and mutations. We started a comparative genomic analysis of fungal TEs using a rigorous annotation strategy. The challenge is to detect *ab initio* TEs and annotate them including nested and degenerated copies. We use the REPET pipeline developed at URGI which efficiently detects and annotates TEs in novel genomes. We will present preliminary results obtained with REPET on *Botrytis cinerea, Sclerotinia sclerotiorum, Leptosphaeria maculans, Tuber melanosporum* and *Blumeria graminis.* Searching for *B graminis* TEs using RepeatMasker against RepBase Update (known TEs) showed that they represent only 10% of this genome whereas REPET analysis showed up to 75%, TEs mainly as novel elements not already described in RepBase, illustrating the interest of such a pipeline.

12. Identification of new appressorium specific regulatory networks in *Magnaporthe grisea*

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Magnaporthe grisea is responsible of one of the major disease on rice, and has emerged as a model for studying fungal-plant interactions. Early stage of its infection process involves specialized cells called appressoria that are required for penetration into host leaves. Since only few appressorium specific transcription factors have been identified, genes differentially expressed in appressoria were highlighted using DNA arrays. Among 900 genes over-expressed in appressoria, 29 encode putative transcription factors (TFs). Bioinformatics analysis confirmed that they encode TF from different classes (Zn2Cys6, BZip, C2H2). Their expression was assessed by qRT-PCR at different developmental stages (mycelium, conidia, appressoria) and during infection of barley leaves. Seven of these genes were highly over-expressed in early stage of infection and appressoria. They have unknown function in fungi and were chosen for further studies. Six deletion mutants were obtained by targeted gene replacement and their capacities to infect their host were tested. A comparison of the transcriptome of wild type and mutant appressoria will be performed to identify the target genes of these transcription factors and understand their role in appressorium development.

13. Functional Characterization of RasGEF and RasGAP genes in *Magnaporthe grisea*

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Ras-guanine nucleotide exchange factor (RasGEF) and Ras GTPase-activating protein (RasGAP) are conserved in eukaryotes and play important roles in different developmental processes. To identify the RasGEF and RasGAP genes functioning upstream from *RAS2* that functions upstream the cAMP signaling and Pmk1 MAPK pathways, we have identified and functionally characterized two putative RasGEF genes (*RGF1* and *RGF2*) and four putative RasGAP genes in *M. grisea*. Deletion of *RGF2* had no obvious defects, while the *rgf1* deletion mutant had pleiotropic phenotype. It was significantly reduced in conidiation and vegetative growth. Only less than 10% of the spores germinated and formed appressoria. However, these appressoria were defective in penetration of onion epidermal cells. The *rgf1* mutant was nonpathogenic in infection assays with rice and barley seedlings. Preliminary analysis indicated that the phosphorylation level of Pmk1 was not affected in the *rgf1* mutant. Exogenous cAMP stimulated appressorium formation in the *rgf1* mutant. In the *rgf1* deletion mutant and transformant expressing the $RGF1^{G108R}$ allele, the intracellular cAMP level was reduced about 2 folds in comparison with that of the wild-type strain. Expression of a dominant active *RAS2* allele in the *rgf1* mutant stimulated the formation of melanized hyphal tips. These data suggested that *RGF1* but not *RGF2* functions upstream of the cAMP signaling pathway and Ras2. We also have generated deletion mutants for three RasGAP genes and RNAi silencing mutants for the fourth one. Phenotype characterization of these RasGAP mutants is in progress and will be present.

14. Comparative functional genomics: Reconstructing the evolution of central carbon metabolism in 15 fungal species

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Molecular networks are the information processing devices of cells and organisms. Networks are remarkably flexible and can re-configure in an adaptive response to perturbation. We seek to understand the mechanisms by which molecular networks accommodate changes by analyzing genomics data through the unifying abstraction of the functional module. Specifically, we focus on evolutionary changes that occur in the organization and regulation of central carbon metabolism in *Ascomycota* fungi. Although central carbon metabolism follows the same general theme in all yeasts, important biochemical, genetic and regulatory variations exist. For example, physiologically yeasts can be classified into respiro-fermentative and respiratory during aerobic growth on glucose. We constructed a genomics platform to study a set of 15 fully sequenced yeast species spanning over 300 million years of evolution. We grow each organism under a variety of conditions (e.g. rich glucose, alternative carbon sources) and along transitions from one condition to another. We measure a variety of phenotypes: growth characteristics, global expression, metabolomics and proteomics profiles. We use our computational algorithms for orthology mapping (SYNERGY) and regulatory network reconstruction (CISPROF), along with novel algorithms, to reconstruct the modules in each extant species, and those that existed in ancestral ones. We then mine those reconstructions for general patterns of how module content and regulation evolves and how changes occur at different molecular levels (transcription, protein, metabolic). Analysis of global expression and metabolite profiles from experiments in which the cells are grown until the glucose is depleted and alternate carbon sources are metabolized revealed both universal (e.g. repression of ribosomal genes) and species-specific (e.g. differential regulation of oxidative phosphorylation) trends. We have reconstructed many of the ancestral modules of this group giving us unique insight into the evolutionary process.

15. Proteome maps of total cell, mitochondrial and secreted proteins of *Aspergillus fumigatus*.

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Aspergillus fumigatus is a ubiquitously distributed saprophytic mould. Upon inhalation, *A. fumigatus* spores germinate, undergo hyphal growth and spread in the lungs causing deadly invasive aspergillosis in immunocompromised patients. In comparison to other fungi, *A. fumigatus* possesses obviously higher stress tolerance and better mechanisms to adapt to the host environment, since this *Aspergillus* species is the prominent cause for such severe lung infections. As a basis for comparative proteomic studies, we established a reference proteome map. Using MALDI-TOF-MS/MS we identified 392 protein spots representing 344 proteins separated on 2D-gels. Proteins involved in primary metabolism, protein synthesis, cell cycle regulation, DNA processing and transcription, cellular organisation and stress response were most abundant. Since mitochondria are involved in many essential processes such as energy metabolism, cellular differentiation and cell death, we established a protocol for the isolation of mitochondria of *A. fumigatus* mycelium and constructed a 2-D reference-gel for this organelle as well. 136 proteins represented by 196 spots have been identified so far. To complete the analysis of the mycelial proteome, we analysed the secreted proteins (secretome) under in vitro conditions. In many cases, proteins secreted by pathogens play an important role for their virulence. For *A. fumigatus* only little information about secreted proteins and their contribution to virulence is available. About 80 different proteins, represented by 272 spots, were detected of which approximately 45% were predicted as secreted proteins by bioinformatic analysis. Proteases and proteins involved in cell wall metabolism were most abundant.

16. The *flbE* gene encodes a novel protein necessary for asexual development in *Aspergillus*

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The asexual spore formation (conidiation) in filamentous fungi is an established model system for studying gene expression differentiation and intercellular communication. The *fluG* and *flbA~E* gens have been identified as early regulatory genes acting upstream of BrlA. In this study, we characterized the *flbE* gene. FlbE shows trans- activation activity in the N-terminus. The mRNA of *flbE* accumulates during the initial stage of vegetative growth only in *Aspergillus nidulans*. The *flbE* mRNA in *Aspergillus fumigatus* started to accumulate in the early phase of vegetative growth, and presents some levels throughout asexual development. The *flbE* deletion mutant shows delayed and reduced conidiation in both *A. nidulans* and *A. fumigatus*. The accumulation of *brlA* and *vosA* (related to spore maturation) mRNAs was delayed during asexual development in the *flbE* deletion mutant of *A. nidulans*. The overexpression of *flbE* causes a fluffy phenotype, hyphal disintegration (autolysis) and delayed expression of *brlA* and *vosA* during development. In summary, FlbE is an essential activator of asexual development and the dosage of FlbE appears to be important for the development in *Aspergillus* species.

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17. cAMP signaling in *Fusarium verticillioides* involves in growth, microconidiation, macroconidiation, germination, virulence, and stress response.
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Fusarium verticillioides is one of the most important fungal pathogens of maize and a producer of mycotoxin fumonisins. In filametnous fungi, cAMP signaling has been implicated in diverse cellular processes, including stress response, metabolism, development, and virulence. However, little is known about its role in the biology, pathogenesis, and secondary metabolism in *F. verticillioides*. In this study, we generated deletion mutants of the adenylate cyclase *FAC1* gene and the *CPK1* gene encoding the catalytic subunit of protein kinase A. The *fac1* and *cpk1* deletion mutants were reduced in radial growth and macroconidiation. Macroconidia were not produced by the *fac1* mutant but the *cpk1* mutant still produced rare macroconidia. The *fac1* mutant also were defective in pathogenesis and germination of microconidiation. Interestingly, the *fac1* mutant was more tolerant to heat and oxidative stress. Reintroduction of the wild type *FAC1* and *CPK1* alleles fully complemented the defects of the corresponding mutants. The *fac1* mutant failed to produce microconidium chains and was reduced in the expression of the *HYD1* and *HYD2* hydrophobin genes, which are known to be associated with the formation of conidium chains. Our results indicate that the *FAC1* gene is required for the production of macroconidia and microconidium chains in *F. verticillioides*. The *fac1* and *cpk1* deletion mutants generated in this study will be used for comparative genomics analysis of cAMP signaling in three closely related *Fusarium* species.

18. Improving gene targeting in *Aspergillus nidulans* for high through-put experiments
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Gene targeting in filamentous fungi has until recently been cumbersome due to difficulties with gene-targeting substrate construction and low efficiencies demand laborious screening and verification of correctly targeted candidates. Recently, progress has been presented to diminish both bottlenecks. Hence, PCR based methods for substrate construction has been presented, and strains designed for efficient gene- targeting have been developed by eliminating the non-homologous end-joining pathway (NHEJ). Nevertheless, to facilitate high through-put gene targeting experiments, further improvements will be desirable. We will present new tools to facilitate easy production of substrates for construction of deletions, point mutations, GFP taggings etc. In addition, since elimination of NHEJ still produces significant background, success rates are typically in the range of 50-90 %, high through-put experiments will still benefit if this efficiency can be further improved. Since the mechanism for the residual random integration is unknown, we have determined its genetic requirements in *Aspergillus nidulans*. Interestingly, the *RAD52* homolog, *radC*, appears to play a crucial role in this type of random integrations indicating that microhomology-mediated end-joining could be a possible integration pathway. To test this model, we are currently investigating the DNA sequences around double strand breaks, which have been repaired in the absence of NHEJ and not by traditional HR. Moreover, since RadC also plays a key role in gene targeting, no straight forward genetic manipulation can be done to channel more gene targeting substrates into the HR pathway. We have therefore evaluated different types of gene targeting substrates to address whether substrate design can influence the gene targeting efficiency in NHEJ deficient strains.

19. Differential gene expression in the Lolium pratense - Epichloë festucae symbiotum during benign seed transmission and stromata formation.
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The Lolium pratense - Epichloë festucae symbiotum is a model system for symbioses of Epichloë and Neotyphodium species (endophytes) with C3 grasses, which receive great fitness benefits. The E. festucae sexual cycle is associated with a mildly pathogenic state, the formation of a stroma on a minority of tilers. To identify genes involved in disease development versus benign plant colonization, expression analysis was conducted using '454' pyrosequencing of mRNA from normal inflorescences and stromata of E. festucae-infected plants. In total, 1734 putative differentially expressed fungal genes were identified. To further examine expression of several of these, fungal mycelium and three tissues from four different L. pratense-E. festucae symbiota were extracted and used in RT-qPCR studies. Delta-Delta-CT analysis of the RT-qPCR data validated significant 454 results ($p \leq 0.05$) in most cases. For example, RT-qPCR showed that sspA, a predicted secreted protein gene, is over 100-fold upregulated in inflorescences versus stromata. This gene, with its native promoter, has been cloned into an expression vector to generate a C-terminal translational fusion with an autoflorescent protein, and introduced into E. festucae. Perennial ryegrass has been inoculated with the transformed E. festucae to observe the expression of sspA in symbio via fluorescence microscopy. Other genes found to be differentially expressed will also be studied by in symbio localization.

20. The Jena genome sequencing and analysis project of the dermatophytic fungi *Arthroderma benhamiae* and *Trichophyton verrucosum*.
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Millions of humans and animals annually suffer from superficial infections caused by a group of highly specialized filamentous fungi, the dermatophytes. In the present study, we set out to reveal and compare the genome sequences of two human pathogenic dermatophyte species, *Arthroderma benhamiae* and *Trichophyton verrucosum*. This approach was launched not only to enlighten common pathogenicity associated traits in these two phylogenetically very closely related dermatophytes, but also to decipher species specific differences in growth behaviour and host preference. Both *A. benhamiae* and *T. verrucosum* are zoophilic species which induce highly inflammatory cutaneous infections in humans such as *Tinea corporis*. In contrast to *T. verrucosum*, *A. benhamiae* is able to complete the sexual cycle and grows well on different media. The 22.5 megabase (Mb) genomes of both human pathogenic dermatophytic fungi were sequenced, the protein- encoding genes identified. Also, we developed an advanced gene-knock out system for *A. benhamiae* making it an ideal model system to understand pathogenicity of dermatophytes.

21. Whole genome annotation and comparative analysis of whiterot fungus Pleurotus ostreatus

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The whiterot fungus *Pleurotus ostreatus* actively degrades lignin—the second most abundant biopolymer on Earth and an important biomass component of wood and agricultural waste. This edible oyster mushroom also degrades a variety of polycyclic aromatic hydrocarbons and has potential for *in situ* bioremediation of contaminated soil. JGI sequenced, assembled, and annotated the haploid *Pleurotus ostreatus PC15* strain with 8.7x sequencing depth coverage of the resulting 34.3 megabase draft assembly. Annotation using the JGI Annotation pipeline yielded over 11,000 gene models. Approximately 97% of over 27,000 sequenced ESTs were mapped to the assembly and supported about one quarter of the predicted gene models. The *P. ostreatus* assembly and gene families were compared with other basidiomycetes including *Phanerochaete chrysosporium, Postia placenta*, and *Laccaria bicolor.*

22. Comparative genomics tools for analysis of six Dothideomycete genomes

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Comparative genomics is a powerful tool for genome annotation and analysis. We recently initiated analysis of six of the Dothideomycete genomes that are publicly available: *Mycosphaerella graminicola, Mycosphaerella fijiensis and Cochliobolus heterostropus C5* all sequenced and annotated at the DOE Joint Genome Institute as well as *Alternaria brassicicola* sequenced at the Washington University School of Medicine, Genome Sequencing Center and annotated by the Christopher Lawrence Lab at Virginia Bioinformatics Institute, Virginia Tech, *Pyrenophora tritici-repentis and Stagonospora nodorum*, both sequenced and annotated by the Broad Institute, MIT. We placed these genomes into a comparative framework equipped with tools to facilitate genome analysis: VISTA genome conservation curves linking genome browsers, gene cluster browser and protein links to homologs in all six genomes, side-by-side comparisons of KEGG pathways and KOG functions, and synteny viewer. JGI community annotation brought biologists behind each of these genomes together to explore common mechanisms of plant pathogenicity and host interactions.

23. Remodeling of global transcription patterns of Cryptococcus neoformans genes mediated by the stress-activated signaling pathways in response to environmental changes

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Human pathogenic Cryptococcus neoformans causing a life-threatening meningoencephalitis sense and respond to harsh host environmental changes occurring during infection. In spite of extensive analysis of central components of the stress-activated signaling pathway, downstream signaling proteins and its network is poorly understood in C. neoformans. Here we employed comparative genomics approach with DNA microarray to explore environmentally regulated genes in C. neoformans. By comparing genome-wide transcription patterns of the wild-type strain with Hog1 MAPK mutants and two response regulator mutants in response to osmotic changes, oxidative damages, and antifungal treatment, we not only gained important insight on global gene expressions patterns for counteracting external stresses, but also elucidated many characterized and uncharacterized stress-related genes in C. neoformans of which expressions are controlled by the stress-activated signaling pathways.

24. Characterization of mating-type mutants of the homothallic ascomycete *Sordaria macrospora*

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The filamentous ascomycete *Sordaria macrospora* is homothallic (self-fertile). In the closely related, heterothallic ascomycete *Neurospora crassa* the mating type of the haploid mating-partners is genetically determined by the mating-type locus, which is present in two different forms (mat a and mat A) in compatible mating partners. The mating type of *S. macrospora* consists of four genes (SmtA-1, SmtA-2, SmtA-3 and Smta-1) sharing similarities to mating-type genes from *N. crassa* mat A- and mat a-strains. These genes code for putative transcription factors (SmtA-1, Smta-1) or proteins without characteristic DNA-binding motifs (SmtA-2, SmtA-3). In this study we analysed the role of the mating-type genes SmtA-1, SmtA-2 and SmtA-3 in *S. macrospora*. Knockout-mutants of all three mating-type genes were generated and analysed. DeltaSmtA-1 and DeltaSmtA-3 mutants are fertile, while a DeltaSmtA-2 mutant is not able to produce mature fruiting bodies and ascospores. Expression of pheromone and pheromone receptor genes was analysed by quantitative Real-Time-PCR analysis. A cross-species microarrays analysis using *N. crassa* arrays was performed to find other differentially regulated genes in the *S. macrospora* mating-type mutants.

25. An initial characterization of ATP-binding-cassette (ABC) transporters of *Grosmannia clavigera*, a mountain pine beetle (MPB) associated pathogen.

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The sapstaining and pine fungal pathogen *Grosmannia clavigera* survives in the toxic lodgepole pine defense environment when it is introduced into the tree by its MPB vector. Constitutive and induced terpenoids and phenolics are the major tree defense compounds. Preliminary work indicated that *G. clavigera*'s ABC transporters were highly induced by pine defense chemicals and may detoxify terpenes and phenolics. We retrieved five transporters from fungal draft genome that we had generated, and used quantitative PCR to determine their expression levels under a variety of growth and stress conditions. One of the transporters was induced only by terpenes, while two of the others were induced by terpenes and by oxidative stress. To characterize the function of highly induced transporter genes we are using complementation with *Saccharomyces cerevisiae* and *Neurospora crassa* single mutants that have deletions for orthologs of *G. clavigera* ABC transporters.

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26. A library-based method to rapidly analyse chromatin accessibility at multiple genomic regions.

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Traditional chromatin analysis methods only test one locus at the time or use different templates for each locus making a standardized analysis of large genomic regions or many co-regulated genes at different loci a difficult task. On the other hand, genome wide high-resolution mapping of chromatin accessibility employing massive parallel sequencing platforms generates an extensive data set laborious to analyse, and is a cost-intensive method only applicable for the analysis of a limited set of biological samples. To close this gap between the traditional and the high-throughput procedures we have developed a method in which a condition-specific, genome-wide chromatin fragment library is produced and then used for locus-specific chromatin fragment analysis. After the establishment and quality control of the library, it can be used for hundreds of loci-specific PCR reactions amplifying with one gene-specific primer and one adaptor-specific primer. The reaction products are analysed by a capillary sequencer rapidly providing accurate numbers for nucleosomal position calculations of several loci. We validated this method by analyzing nucleosomal positioning in the well characterized nitrate-responsive *niiA-niaD* bidirectional promoter of the ascomycete fungus *Aspergillus nidulans.* Additionally, we have used the condition-specific libraries to study nucleosomal positioning at two different loci, the promoters of the general nitrogen regulator *area* and the regulator of secondary metabolism, *aflR*.

27. Proteomic survey of secreted enzymes from various fungi

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We are using enzyme assays and high throughput proteomics to investigate the glycoside hydrolase activities of various fungi and establish the qualitative expression profile of these enzymes under different growth conditions. The fungi were selected based on three criteria. The invariant criterion was the existence of a genome sequence (currently or in the near future), which is preferable for applying the shotgun proteomics approach. Fungi that have a known or anticipated large complement of glycoside hydrolase genes were preferred. Finally, we attempted to represent some of the diversity across the fungal kingdom. The fungi include: *Thielavia terrestris, Aspergillus niger, Aspergillus carbonarius, Fusarium oxysporum, Trichoderma atroviride, Postia placenta* and *Phycomyces blakesleeanus*. These fungi were grown on complex plant biomass sources or glucose as a control. High throughput liquid chromatography-tandem mass spectrometry techniques were employed to detect and identify secreted proteins in the broths from these cultures. Enzyme assays for specific glycoside hydrolase activities were also performed to check for corroboration with the identifications made by proteomics. The results for the two fungi completed to date show different patterns of glycoside hydrolase expression that may contribute to a better understanding of the determinants of efficient biomass hydrolysis.

28. The *Aspergillus* Genome Database (AspGD), a curated database of *Aspergillus* gene, protein, and genomic sequence information for the fungal research community.

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We have recently obtained funding to create a resource for the *Aspergillus* research community, AspGD, which will be a multispecies genomics database modeled on the *Candida* and *Saccharyomyces* Genome Databases. Our goal is to facilitate research on Aspergilli and on other medically and economically important fungal pathogens by providing a first-stop reference for *Aspergillus* genomics and molecular biology, with up-to-date, high-quality, curated scientific information and web- based research tools. We will implement an optimized annotation pipeline across all *Aspergillus* genomes, to maintain a set of current and consistent gene boundary annotations, and will incorporate links between genomes based on orthology and synteny data. Gene function, localization, and mutant phenotype annotations will be manually curated from the *Aspergillus* literature. We will provide web-based tools for sequence visualization and retrieval, and for analysis of sequence information and gene product annotations. All of the data in AspGD will be freely available to the public from http://www.aspgd.org/. In addition, we will maintain a colleague registry by which *Aspergillus* research community members may share contact information and research interests, to facilitate collaboration. We strive to be responsive to the needs of the research community, and we welcome your feedback and suggestions.

AspGD curators may be reached by email at aspergillus-curator@genome.stanford.edu AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

29. Horizontal gene transfer of secondary metabolite genes from bacteria to fungi

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Almost all fungal nonribosomal peptide synthetases (NPSs) arose from one ancestral gene, and the two major groups of fungal polyketide synthases (PKSs) each arose from one gene also. In contrast, a small number of NPSs and PKSs (including ACV synthetases for penicillin-type compounds) have separate origins; they group within various clades of bacterial genes. One of these, NPS7/PKS24, is a functionally uncharacterized hybrid gene from Cochliobolus heterostrophus, and has orthologs in two other sequenced fungi: Aspergillus niger and Chaetomium globosum. Questions about its origin and evolution include: Which bacterial lineage is the source, and was it an endosymbiont or free-living? Was the gene inherited as a hybrid, or was it the result of gene fusion? Was it subsequently inherited vertically or horizontally among lineages of fungi? If it has been inherited vertically since its transfer from bacteria, what selection processes may account for its highly discontinuous distribution within and among classes of ascomycetes? Are the gene lineages under purifying or diversifying selection, indicating either conservation or change in enzyme function? Have their domain structures changed, including loss of functionality, indicating changes in function? To address these questions, DNA sequence from many species in the three fungal genera will be analyzed. A degenerate PCR primer approach has resulted in NPS/PKS amplicons from eight Aspergillus spp. and nineteen Chaetomium spp. Preliminary results will be presented that address some of these questions.

30. Investigation of genes differentially transcribed in *Aspergillus fumigatus* in response to human neutrophils.

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Aspergillus fumigatus is the predominant cause of invasive aspergillosis in severely immunocompromised patients. Mortality rates of invasive aspergillosis are significantly higher compared to other systemic mycoses due to the difficulty in early diagnosis coupled with high rates of therapy failure. In order to develop new diagnostic methods and improve therapy, an in-depth study on the pathobiology of this fungus is essential. We compared the transcriptome profiles of conidia and hyphae of *A. fumigatus* challenged with neutrophils from normal donors as well as donors with chronic granulomatous disease (CGD). The neutrophils from CGD patients are defective in the production of reactive oxygen species. Such comparisons showed an array of genes that were differentially transcribed in response to neutrophils. We chose 4 genes (2 transcription factors, a hypothetical protein and a nitroreductase family protein) that were up-regulated in conidia and/or hyphae in response to neutrophils from normal or normal and CGD patients to evaluate their function in *A. fumigatus*. A gene deletion approach was used for the functional study. The deletion constructs of these 4 genes were transformed into *A. fumigatus*via *Agrobacterium tumefaciens* mediated transformation protocol. Transformants with homologous recombination of the deletion construct at the target site were identified using Southern analysis. Preliminary observations showed that the growth rate of the mutants as well as the morphological features of colony and conidial structures, including the size and pigmentation of conidia, was similar to those of wild type. This suggested that deletion of these genes had no effect on their basic metabolism. Phenotypic characterization of these mutants is currently underway.

31. *Fusarium verticillioides*: Talking to friends and enemies

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Fusarium verticillioides is both a symptomless endophyte and a pathogen of maize. At some point, the fungus may synthesize fumonisins which have been linked to a variety of animal diseases including cancer in some animals. In order to minimize losses due to contaminated food or feed, we are working to understand the processes involved in fumonisin synthesis as well as how *F. verticillioides* interacts with maize and other fungi that infect maize. Fungi known to interact with *F. verticillioides* in maize include *F. graminearum*, *Aspergillus flavus*, *A. niger*, and *Penicillium oxalicum*. One important question we are exploring is: How do these fungi communicate with each other on maize? We hypothesize that small organic acids commonly associated with these fungi, and which diffuse in advance of infecting hyphae, may play a role in communication. In this study, we exposed *F. verticillioides* to fusaric, kojic, citric and oxalic acid and examined transcriptional changes by microarray analysis. We identified a number of up- and down-regulated genes of which some were similarly affected by all four acids as well as others that were uniquely affected by each acid. The transcriptional differences observed clearly show that *F. verticillioides* is differentially responding to the different acids. This supports our hypothesis that these acids serve as signaling molecules informing *F. verticillioides* of its immediate neighbors which enables the endophyte to tailor an effective response.

32. *Verticillium* comparative genomics – understanding pathogenicity and diversity.

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Verticillium dahliae is the primary causal agent of Verticillium wilt that causes billions of dollars in annual losses worldwide. This soil-borne pathogen exhibits high genetic plasticity, colonizing numerous plant hosts in diverse ecological niches. We employed comparative genomics with *V. dahliae* and a related species, *V. albo- atrum*, that differs in host range. A 7.5X assembly of the 33.8 Mb genome of a *V. dahliae* lettuce isolate, plus a 4X assembly of an alfalfa isolate of *V. albo-atrum* are now publicly available via the Broad Institute. ~37,000 ESTs from three *V. dahliae* cDNA libraries were generated, and both genomes annotated. Comparative analyses revealed four major *V. dahliae*-specific regions. Each spans ~300 kb and is enriched in repetitive DNA and transposons. Corresponding ESTs in these regions confirmed *V. dahliae*-specific gene expression. We are currently examining these regions to gain insight into diversity, pathogenicity, and other aspects of *Verticillium* spp. biology.

33. Fungal intron evolution: why small genome can have many introns

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Previous phylogenetic intron studies have focused on individual genes and intron locations. The best whole genome studies used only 30 genes. Here we used a statistical approach instead. We analyzed exon number and intron length from whole genomes of seven Basidiomycota, seven Ascomycota, one Zygomycota, and one Chytridiomycota species. A whole genome phylogenetic tree was constructed using 288 proteins which agree with previous publications. Our exon number analysis indicates that the ancestor of fungi have large numbers of exons (from 5.8 to 7.2). Sporobolomyces roseus is most related to Ustilago maydis based on our phylogenetic tree. Both have small genomes but they differ dramatically in the number of exons per gene (S. roseus with an average 7.2 and U. maydis 1.7). The large number of exons per gene in S. roseus correlates with lack of reverse transcriptase (RT) foot-prints in the genome and lack of symptoms of RT-mediated intron loss. We also found that the number of genes in each genome is directly correlated with the genome size (slope = 4167 nucleotides per gene). We identified a clear difference between Ascomycota and other fungal phyla in the pattern of intron loss with the exception of U. maydis, whose pattern resembles that of the Ascomycota yeast Pichia stipitis. We also identified a phylogenetically divergent effect of RT on exon numbers per gene. RT correlates with intron loss of Ascomycota but correlates with intron gain in Basidiomycota. In addition we identified RT as a major contributing factor to fungal genome size. Finally we found that the number of exons per gene within the same genome differ between genes of different degrees of conservation, and there is a direct correlation between the number of exons from genes conserved in all 16 genomes and the difference between between this and that of genes that are specific to each species, with a coefficient of 0.5 to 0.54. So we call this The Half Difference Rule.

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34. A comparison of predicted highly expressed genes in fungal genomes.
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The genes that are highly expressed in an organism often reflect its predominant lifestyle and its major metabolic activities in its natural habitat. Comparing genes that are predicted to be highly expressed in different species provides an opportunity to correlate functional gene classes with particular lifestyles, and to identify potentially important functions within a species, or group of organisms, that were not previously recognized as such. This technique has been applied widely in prokaryotes. The number of recently completed fungal genomes now allows a similar, systematic comparison across the fungal kingdom. In order to predict highly expressed genes in fungi, we scan individual genomes for genes that display a strong codon bias compared to the average gene, and which are similar in codon bias to ribosomal protein genes, translation elongation factor genes, and cytoskeletal genes, which are highly expressed in most species. The resulting gene list is then combined with KEGG Orthology functional classifications to assess the importance of individual cellular components, processes, and metabolic pathways in each fungus. Many of the results to date are consistent with the known, prominent metabolic activities of the organisms that we have examined. For example, a high percentage of genes involved in glycolysis and fermentation are predicted to be highly expressed in anaerobic species, while many TCA cycle and oxidative phosphorylation genes are predicted to be highly expressed in facultative or aerobic species. On-going work compares the predicted highly expressed genes in species with varied lifestyles (e.g. pathogens vs. nonpathogens) and from different taxonomic groups (e.g. Basidiomycetes vs. Ascomycetes).

35. A high resolution genetic map for *Coprinus cinereus*
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Coprinus cinereus (also known as *Coprinopsis cinerea*) is an important model system for analyses of multicellular development and the meiotic process. It is also one of only a small number of fungi in which the assembled genome extends telomere to telomere for the majority of the chromosomes. The total map distance was estimated to be 1300 cM based on chiasma frequency and 1346 cM based on RAPD markers. However, both the cytological evidence and the RAPD map suggested that recombination rates are not uniform across the genome. Since simple sequence repeat (SSR) markers have defined physical locations, we utilized 147 evenly distributed polymorphic SSRs plus 4 additional polymorphisms to construct a genetic map. We designed conditions for high throughput amplification of DNAs in 96 well format and subsequent multiplexed fragment analysis. We obtained evidence for both “hot” and “cold” regions for recombination as predicted from the cytological evidence and the RAPD map. This unequal distribution of reciprocal exchanges along the chromosomes has important implications for meiotic chromosome shape and the degree of linkage exhibited by adjacent markers. Since the genetic map of many fungal species can be expressed as 100cM X N (the haploid chromosome number), the *C. cinereus* pattern of a single genetic exchange/chromosome arm may be widespread among fungi.

36. Development of Chrysosporium lucknowense C1 as a commercial protein production platform: Exploration and exploitation of its genome.
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Several filamentous fungi can secrete large amounts of protein into the growth medium and are therefore commonly used as hosts for the production of industrial enzymes. We have developed the ascomycetous fungus *Chrysosporium lucknowense* C1 for protein production on a commercial scale as an alternative to well known fungi like *Aspergillus niger* and *Trichoderma reesei*. Strain and process improvement strategies of the original C1 isolate resulted in strains that are able to secrete large amounts of a complex mixture of (hemi-)cellulases. Additionally, these strains show a strong reduction in culture viscosity as result of a morphology change. The low-viscosity property allows for growing C1 to high density in fermenters, yielding very high protein production levels. Recently, the sequencing and automated annotation of the C1-genome was finished. Genome mining revealed an impressive enzymatic potential. Especially, the repertoire of genes encoding plant biomass hydrolyzing enzymes appeared overwhelming. Currently, this knowledge is being exploited: C1(hemi-) cellulases obtained by (selective) over-expression and by purification are tested for saccharification of (lingo-) cellulosic substrates. These studies provided leads towards the development of enzyme mixtures that will efficiently and cost-effectively convert residual biomass to fermentable sugars. An overview will be given covering the exploration and the exploitation of the C1-genomic potential in order to develop C1 as a commercial protein production microorganism.

37. Excavating the adaptive palimpsest: acquisition and evolution of ecological function in fungal genomes.
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Fungi, like prokaryotes, can obtain peripheral metabolic pathways through horizontal transfer of gene clusters. Subsequent genomic evolution may be characterized by rearrangement and loss of the genes that make up these clusters and functional diversification of the constituent gene families. We examined peripheral metabolic gene cluster evolution in basidiomycete and ascomycete lineages and evaluated evidence of functional diversification and positive selection that may reflect fungal niche adaptation at the interface between genome and environment.

38. Subtilisin-like (SUB)- and metallo (MEP)-protease genes as putative pathogenicity factors for *Trichophyton tonsurans* (TT) and *Trichophyton equinum* (TE)

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Secreted exoproteases are utilized by dermatophytes for the liberation of nutrients and the disruption of host protective barriers. Given their high degree of sequence similarity and distinct host niches, the closely related anthropophile TT and zoophile TE represent a model-system by which to examine genes that account for differences in host selection. Fungal spores of TT (n=5) and TE (n=4) were inoculated into keratin media and duplicate samples harvested at 4, 7, 10, 12, and 14 d. Total RNA was isolated and qRT-PCR was performed to analyze transcript expression in 7 SUBs and 5 MEPs. Initial expression of SUB7 and MEP2 was 121x and 19x higher, respectively in TT vs. TE. In contrast, SUB1 was significantly higher in TE ($p<0.01$) vs. TT with a similar trend in MEP3 ($p=0.056$). Expression of SUB5 in TT was negligible over 14 d whereas levels in TE appeared to increase, reaching an average 103x higher vs. TT. Similarly, SUB6 transcript remained low in TE while increasing steadily in TT, reaching a 62x increase over TE. SUBs 2, 3, and 4 each revealed similar expression patterns between spp., with the magnitude of expression consistently higher in TT vs. TE. Levels of MEP 4 did not vary in either spp. and MEPs 1&5 were negligible throughout the study. The variance observed in the expression of SUBs 1, 5, 6, 7 and MEPs 2 ,3 suggests that these genes may be contribute to host selection.

39. Whole-genome annotation of the potential biodiesel feedstock *Mucor circinelloides* and its comparison with other "Zygomycota"

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The zygomycote *Mucor circinelloides* (or "MC") is a candidate feedstock for biodiesel because it can accumulate large amounts of suitable lipids, and has a track record of both biotechnological manipulation and large-scale fermentation. Also of interest are its photoresponsiveness, carotenogenesis, dimorphism, and opportunistic pathogenicity. To compliment this fungus' rich history of microbiological and molecular studies, the genome was sequenced to 9X depth and assembled into 23 scaffolds totaling 37 Mbp, the smallest zygomycote genome sequenced so far. Using the JGI Annotation pipeline we predicted ~11,000 protein-coding genes, the lowest gene count in any zygomycote so far. Out of ~4,000 expressed genes , one third is potentially light- specific, another third is dark-specific, and the rest is transcribed under both light and dark as shown by analysis of > 27,000 ESTs extracted from light and dark cultures.. 61% of MC proteins contained Pfam domains, a proportion much higher than seen in the 2 other sequenced zygomycota (*Phycomyces blakesleeanus* or "PB" and *Rhizopus oryzae* or "RO"). 82 domains were found in MC but absent from both PB and RO. 95% of MC proteins align with those of PB or RO while on the DNA level only 41% and 34% of the MC genome aligned with RO and PB respectively. Analysis of gene clusters in the 3 zygomycota revealed 109 clusters that consist of 2 or more MC proteins but no PB or RO members. These include a cluster of 10 proteins with F-Box domains, another of 8 proteins with zinc-finger domains.

40. Proteomic analysis of *Magnaporthe oryzae* during infection structure development

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Rice blast disease is caused by the filamentous Ascomycetous fungus *Magnaporthe oryzae* and results in significant annual rice yield losses worldwide. Infection by this and many other fungal plant pathogens requires the development of a specialized infection cell called an appressorium. The molecular processes regulating appressorium formation are incompletely understood. We analyzed protein profiles during spore germination and appressorium formation. Using liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS), we identified a total of 553 proteins from intact conidia and conidia forming germtubes and appressoria. Approximately 50% (285) of the identified proteins were common to all 3 tissue types and mainly represented proteins involved in primary metabolism. In addition, proteins required for the response to oxidative stress were over-represented and proteins for transcriptional regulation under-represented. Most of the 45 proteins which were uniquely identified in appressorium were functionally unknown but enriched with putative secreted proteins including cell wall modifying enzymes. We functionally characterized a gene encoding the most abundant protein, which was highly expressed transcriptionally throughout appressorium morphogenesis. The knock-out mutant showed reduced growth on non- favorable carbon sources and other defects. Overall, little correlation was found between protein and previously reported gene expression levels suggesting wide-spread posttranscriptional processes regulate infection structure development in *M. oryzae*.

41. Construction of a Gene Replacement resource for *Aspergillus fumigatus* using MultiSite Gateway® Pro Technology.

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We present the construction of a knock-out resource for *Aspergillus fumigatus* available through the PFGRC's Gateway® Entry Clone Resource. A three-fragment MultiSite Gateway® Pro method was used to assemble the *pyrG* selectable marker between flanking chromosomal regions. The marker was sequence verified and a total 70 constructs were confirmed by PCR. Four of the verified constructs were amplified and transformed into chemically competent *A. fumigatus* CEA10 KU80-Delta-pyrG protoplasts and selected for *pyrG+* phenotype. All of the transformations yielded between 3 and 10 viable colonies, indicating chromosomal insertion of the replacement constructs. After re-isolation, 70% of colonies survived. Chromosomal deletions were verified by isolating DNA and performing multi-Plex PCR reactions to confirm the presence of the *pyrG* marker and the correct insertion of the replacement construct. At least one transformant from three loci was confirmed. All reactions were performed at volumes that would allow high-throughput implementation of the method. The overall efficiency of mutagenesis was 75%. The PFGRC is making this resource available to the research community free of charge as individual clones at its website pfgrc.jcvi.org. The resource is funded by NIAID.

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42. Cyberinfrastructure for *Fusarium* (CiF).

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The rapidly increasing number of genome sequences from diverse fungal species and expanding phylogenetic data necessitate highly integrated informatics platforms to adequately support the use of these resources for studying fungal biology and evolution. The long-term goal of CiF (http://www.fusariumdb.org) is to support the archiving, integration and utilization of data from diverse areas of *Fusarium* research, ranging from genomics, phylogenetics, and population genetics to epidemiology. Via a genome browser, genome sequences and features of four *Fusarium* species and characteristics of selected gene families and functional groups can be examined. The CiF will also support identification of *Fusarium* isolates by archiving comprehensive phylogenetic data covering the known diversity of the genus, including those currently archived in the *Fusarium*-ID database, in a searchable format. This new identification tool will allow users to download datasets, including alignments.

43. Absence of cytosine methylation in *Mycosphaerella graminicola* correlates with Repeat Induced Point mutation signatures in its *Dim-2* homologue.

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A *de novo* search for repetitive elements in the genome sequence of wheat pathogen *Mycosphaerella graminicola* identified a family of repeats containing a DNA methyltransferase gene, which is a homologue of the *Neurospora crassa Dim-2* gene. A total of 31 *MgDim-2* sequences were identified, all of which carried signatures of Repeat Induced Point mutations (RIP). All *MgDim-2* copies were sub-telomeric in location, except for one copy on chromosome 6. Synteny of this non-telomeric *MgDim-2* region to a closely related fungus, *M. fijiensis*, implied that the copy on chromosome 6 was the original sequence that was amplified. Amplification of the *MgDim-2* gene in 14 other *M. graminicola* isolates from various geographical regions was substantiated by Southern analysis. However, an *in silico* search for *MgDim-2* homologues identified a single, unRIPed copy in eight ascomycete genomes, suggesting that the amplification event might be specific to *M. graminicola*. A genome-wide assay by ESI-MS/MS revealed that cytosine methylation was present in *M. fijiensis* but absent in *M. graminicola*, as expected if the *MgDim-2* gene is inactivated. Our results indicate that amplification of the single-copy *MgDim-2* gene made it susceptible to RIP, which might be responsible for the complete lack of cytosine methylation in *M. graminicola*.

44. Dissection of polyketide biosynthesis pathways in *Aspergillus nidulans*

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Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, mln@bio.dtu.dk Genome sequencing and subsequent annotation of several *Aspergillus* genomes has revealed the presence of a large number of previously unknown putative gene clusters. Many of the clusters contain an interesting class of multi domain enzyme called polyketide synthases (PKS). The synthases are able to catalyze the polymerization of acetyl and propionyl subunits into large molecules that are subsequently modified by neighboring enzymes encoded within each cluster. The result is the formation of complex molecules with a high degree of structural diversity and often a very specific biological activity. These activities have been applied in a range of pharmaceuticals such as antibiotics, cholesterol lowering agents and anti cancer agents. Although, a few polyketide biosynthesis pathways have been elucidated, most clusters still remain to be mapped and characterized. Moreover, very little is known about how individual tailoring enzymes coordinate their activities. In the present study, we use a combination of reverse genetics and chemical analyses to identify the products of uncharacterized polyketide gene clusters in *Aspergillus nidulans*. This is specifically done by a combination of promoter replacements and gene deletions. For selected clusters we tag genes with fluorescent proteins to asses the spatial and temporal coordination of the biosynthesis. Examples of this approach are presented.

45. Production of glycoside hydrolases for functional annotation

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Glycoside hydrolases (GH) are a diverse group of enzymes with an essential role in the deconstruction of lignocellulosic biomass. Functional characterization of GH enzymes critical for plant biomass deconstruction would provide a valuable resource for renewable fuel production. Our goal in this project was to identify all GH enzymes in the genome of *Aspergillus niger* ATCC1015 and produce purified proteins suitable for characterization by *in vitro* enzyme activity assays. A comparative genomics approach was used to identify the complete genomic set of GHs and classify target by family. These analyses indicate that *A. niger* ATCC1015 contains 236 hydrolase genes, with representatives from 49 families. The structural annotation was refined for a subset of targets to enable cloning and protein production. Fifty targets were cloned into vectors for protein expression in *E. coli* and/or the methylotrophic yeast *P. pastoris*. Protein production in *P. pastoris* was the preferred route and resulted in a 50% success rate for production of expressed and soluble protein. A number of these enzymes have been purified and are being characterized for substrate specificity. These results will lead to improved functional annotation of this enzyme family and to the discovery of novel glycoside hydrolase activities.

46. Analysis of the secretome of the basidiomycete *Coprinopsis cinerea*

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Coprinopsis cinerea is a saprophytic model fungus for the basidiomycetes that can easily be cultured in the laboratory on artificial medium. The genome of the fungus has been sequenced by the Broad Institute offering in silico access to sequences of proteins encoded in the chromosomal DNA. Using a protein database for peptide searches, we analyse the secretome of the fungus in relation to cultural age and to localization of the secreted proteins (free in culture medium, cell wall attached). Many of the secreted proteins are unique to age and/or localization.

47. Withdrawn

48. Proteome maps of total cell, mitochondrial and secreted proteins of *Aspergillus fumigatus*

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Aspergillus fumigatus is a ubiquitously distributed saprophytic mould. Upon inhalation, *A. fumigatus* spores germinate, undergo hyphal growth and spread in the lungs causing deadly invasive aspergillosis in immunocompromised patients. In comparison to other fungi, *A. fumigatus* possesses obviously higher stress tolerance and better mechanisms to adapt to the host environment, since this *Aspergillus* species is the prominent cause for such severe lung infections. As a basis for comparative proteomic studies, we established a reference proteome map. Using MALDI-TOF-MS/MS we identified 392 protein spots representing 344 proteins separated on 2D-gels. Proteins involved in primary metabolism, protein synthesis, cell cycle regulation, DNA processing and transcription, cellular organisation and stress response were most abundant. Since mitochondria are involved in many essential processes such as energy metabolism, cellular differentiation and cell death, we established a protocol for the isolation of mitochondria of *A. fumigatus* mycelium and constructed a 2-D reference-gel for this organelle as well. 136 proteins represented by 196 spots have been identified so far. To complete the analysis of the mycelial proteome, we analysed the secreted proteins (secretome) under in vitro conditions. In many cases, proteins secreted by pathogens play an important role for their virulence. For *A. fumigatus* only little information about secreted proteins and their contribution to virulence is available. About 80 different proteins, represented by 272 spots, were detected of which approximately 45% were predicted as secreted proteins by bioinformatic analysis. Proteases and proteins involved in cell wall metabolism were most abundant.

49. Functional characterization of putative determinants for *Aspergillus fumigatus* sexual development

Szewczyk, E. and Krappmann, S.

Aspergillus fumigatus has been classified for decades as deuteromycete with no described sexual cycle. However, recent observations of chosen isolates cultivated for prolonged time in confined conditions resulted in identification of fruiting bodies, fertile sexual progeny and meiotic gene recombination (O'Gorman *et al*., 2008). The existence of cryptic sexuality in this species has been proposed before based on genomic and genetic analyses revealing presence of the mating type loci (*mat1-1* and *mat1-2*) and many putative genes orthologous to recognized determinants of fruiting body formation in the fertile species *Aspergillus nidulans*. The product of its *mat1-2* gene was shown to be functional in *A. nidulans* (Pyrzak *et al*., 2008) and here we demonstrate functionality of *mat1-1* gene product. The orthologue of the crucial positive transcriptional regulator of sexual development in *A. nidulans*, the *nsdD* gene, is also present in the *A. fumigatus* genome. It is functional in *A. nidulans* supporting cleistothecium formation upon overexpression. Moreover, we show that high-level expression of NsdD in the endogenous host *A. fumigatus* alters hyphal development and morphology triggering formation of coiled hyphae. Deletion of *nsdD* gene in *A. fumigatus* causes slight inhibition of growth with no significant effect on asexual conidiation. However, conidiation is abolished when long chain fatty acids are used as a sole carbon source. The deletant strain also shows significant sensitivity to osmotic and cell wall stresses. Accordingly, our data indicate an involvement of a sexual cycle regulator in cell wall maintenance and might shed light on development of sexual structures in *Neosartorya fumigata*.

50. A Database of Fungal Protein Clusters for Comparative Genomics.

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The Fungal Protein Cluster Database (FPC-DB) enables users to quickly locate information about the phylogeny and functional categories of fungal proteins and gene families and present the information in a number of comparative views. The proteins from fungal whole-genome sequencing projects are annotated with InterPro and Gene Ontology terms using an automated functional classification server previously developed in our group. The proteins are then clustered into putative gene families. Each protein cluster includes multiple sequence alignments, phylogenetic trees and a summary of functional categories found in the cluster. Users can perform queries using protein IDs, protein functional categories, and species names as search terms. The results are displayed in a variety of forms that allow users to compare the occurrence of gene and their functional categories between species. Users can also view a detailed page for each protein cluster and simultaneously view a phylogenetic tree and a matrix of functional categories. Comparative views enable users to directly compare gene family content between species to identify changes in gene family size between species.

51. A genomic survey of transporters and their expression in a grass-endophyte symbiosis reveals putative key genes in nutritional cross talk.

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Epichloë endophytes are mutualistic fungal symbionts of cool-season grasses, known to confer growth advantages to their hosts. As a step towards understanding grass- epichloë interactions, we have focused on identifying transporter genes specifically expressed in this symbiotic association.
The genome of *Epichloë festucae* has been sequenced and assembled with a near complete coverage. Using a bioinformatics approach based on nearest neighbor method to compare against the transporter classification database (www.tcdb.org), we have identified the putative transporter complement in the *E. festucae* genome. The same approach was used to analyze seven other ascomycete genomes and identify their putative transporters. The respective transporter classifications for the predicted genes were also determined. Comparison of the transporter profiles across the ascomycete fungal genomes revealed gain and loss of certain transporter subfamilies in *E. festucae* genome that may have contributed to the evolution of this species.
To gain insights to the epichloë-plant interaction we identified the putative plant transporters from 17,000 transcript assemblies from tall fescue. We compared the expression levels of transporter gene families in both plant and fungus to identify symbiosis related genes. Comparing in planta expression levels to those in cultured mycelia, we identified a number of fungal transporters significantly up-regulated in the symbiosis. Expression levels of plant transporters in shoots and roots of endophytic vs non-endophytic plants indicated major changes in response to the fungus across a number of transporter gene families.
Results of our genome and expression analysis provide a basis for selecting key genes for further functional analysis.

52. The draft genome sequence for *Eremothecium cymbalariae*, a close relative of *Ashbya gossypii*.

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E. cymbalariae is a close relative of *Ashbya gossypii*, whose genome sequence has been established previously (Dietrich et al., 2004). Both species are filamentous fungi that show bifurcational (Y-shaped) tip growth. In contrast to *A. gossypii E. cymbalariae* generates an aerial mycelium with hyphae that form sporangia at their ends. Spores lack appendices with which spores of *A. gossypii* stick together in bundles. *E. cymbalariae* hyphae lack chitin accumulation at septa characteristically found in filamentous fungi. To explore these differences on a genomic level we have established a draft genome sequence for *Eremothecium cymbalariae*. We obtained one million reads with an average read length of >400 nucleotides using the new generation Roche 454 Titanium sequencing, which represents a 40x coverage of the genome. Assembly of the data currently produces 209 contigs (largest is 509kb, average contig size is 45kb) with a total length of 9.7 megabases, which is slightly larger than the 9.2 Mb for *A. gossypii*. We found orthologs of close to 4500 *A. gossypii* genes and 170 tRNAs. Of the 250 *A. gossypii* genes that were not found in *E. cymbalariae* 88 correspond to *A. gossypii* genes without homology to *S. cerevisiae*. In addition, several genes in *E. cymbalariae* share homologs with *S. cerevisiae* or *Kluyveromyces lactis*, but are not present in *A. gossypii*. Most *E. cymbalariae* genes are within blocks of synteny with *A. gossypii* genes. Using this syntenic gene order we identified seven centromeres in *E. cymbalariae*, indicating 7 chromosomes as found in *A. gossypii*. Interestingly, *E. cymbalariae* has two functional alpha factor encoding genes with four and three mature peptides in the pre-pro-protein, while in *A. gossypii* one is apparently truncated and the other one only encodes a single pheromone peptide. We will present phenotypic comparisons of *E. cymbalariae* and *A. gossypii* as well as our current status of the genome assembly.

53. Comparative transcriptomics analysis of glycerol metabolism in Aspergilli

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Glycerol is becoming of considerable importance in industrial fermentation processes as it is a major by-product from biodiesel production; thereby it represents an inexpensive carbon source for bio-based production of chemicals. It is catabolized by a broad range of microorganisms such as *Aspergillus* species at fairly high consumption rates. In *S. cerevisiae*, the transcriptional activator Adr1 regulates the expression of genes required for glycerol, ethanol and fatty acid utilization. In order to identify the transcriptional regulation of glycerol metabolism in *Aspergillus*, we performed triplicate batch fermentations on *A. oryzae*, *A. niger* and *A. nidulans* with glucose or glycerol as carbon sources. Protein comparisons of all three species and cross analysis with gene expression data resulted in the identification of 88 genes having a conserved response across the three Aspergilli. With a promoter analysis of the up regulated genes we identified a conserved binding site for a putative regulator to be 5'-TGCGGGG-3', a binding site that is similar to that of Adr1 in yeast. This binding sequence was over- represented on promoter regions of several genes primarily involved in ethanol, glycerol, fatty acid, amino acids and formate utilization. We therefore propose that Adr1 is likely to be cross species conserved among *S. cerevisiae* and *Ascomycetes* species. Subnetwork analysis was applied to uncover major changes occurring in the metabolism showing that the most active pathway in *A. oryzae* seems to be the one using glycerol dehydrogenase and glycerone kinase to produce dihydroxiacetone phosphate. We thus demonstrate that cross species evolutionary studies within filamentous fungi using comparative genomics and transcriptomics are a powerful tool for dissecting regulatory systems.

54. FlbC is a C_2H_2 transcription factor required for conidiation in *Aspergillus nidulans*

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BrlA is a key transcriptional activator of asexual development in *Aspergillus nidulans*. The fluffy low *brlA* expression (FLB) loci are all required for normal *brlA* expression. We characterized the *flbC* gene, and found that it encodes a potential transcription factor with a C_2H_2 zinc finger domain at the C-terminus and an activation domain at the N-terminus. FlbC is highly conserved in filamentous fungi. The level of *flbC* mRNA during the *A. nidulans* life cycle increases at 12 h of vegetative growth and late sexual development, and decreases at the late phase of asexual sporulation. The deletion of *flbC* results in delayed conidiation and altered accumulation of *brlA* and *vosA* mRNAs. While overexpression of *flbC* inhibited conidiation in air- exposed culture condition, and it causes different pattern of *brlA* and *vosA* mRNAs compare to control during asexual development. Moreover, OE*flbC* resulted in forced accumulation of *brlA*, *abaA*, *vosA* mRNAs in liquid submerged culture, but it can not express the accumulation of *wetA* mRNA. These results suggest that FlbC is a potential transcriptional activator of asexual development, and overexpression of *flbC* causes hyper-vacuolation and forced accumulation of *vosA* through expression of *brlA* and *abaA* mRNAs in *A. nidulans*.

55. A protein function classifier for fungi.

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The Gene Ontology (GO) is a controlled vocabulary of terms to describe protein functions. It also includes a hierarchical description of the relationships among the terms in the form of a directed acyclic graph (DAG). Several systems have been developed that employ pattern recognition to assign gene function, using a variety of features, including sequence similarity, presence of protein functional domains and gene expression patterns, but most of these approaches have not considered the hierarchical structure of the GO. The DAG represents the functional relationships between the GO terms, thus it should be an important component of an automated annotation system. We have developed a Bayesian, multi-label classifier that incorporates the relationships among GO terms found in the GO DAG. We trained the classifier using more than 6000 annotated proteins from fungi obtained from public databases. A comparative analysis of our method to other previously described annotation systems shows that our method provides improved annotation accuracy. More importantly, our method enables the classification of significantly more GO terms to more proteins than other classifiers.

56. Heteroresistance to fluconazole in *Cryptococcus gattii* strains

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Heteroresistance of *Cryptococcus neoformans* to azole drugs, reminiscent of methicillin heteroresistance in *Staphylococcus aureus*, was reported from cases of recurrent cryptococcosis during azole maintenance therapy. In this study, we analyzed 75 clinical and environmental *C. gattii* strains of both serotypes B and C that had been isolated from different geographic areas. All strains of *C. gattii* manifested heteroresistance and the lowest level of heteroresistance to fluconazole in *C. gattii* was significantly higher (>16 ug/ml) than that observed in strains of *C. neoformans* (~4 ug/ml). The environmental isolates from Australia (VGII) exhibited the highest level of heteroresistance while those from India (VGI) expressed the lowest level of heteroresistance. Isolates from Vancouver Island (VGII), were found to show intermediate level of heteroresistance to fluconazole. The levels of drug resistance in representative isolates of these three classes were raised by step-wise increase of the drug concentration and were analyzed for loss of resistance to fluconazole by repeated transfers in drug free media. Strains exhibiting the highest level of heteroresistance required the most transfers before reverting to the original level of heteroresistance. Importantly, the strains heteroresistant at higher levels of fluconazole were significantly more virulent than the strains heteroresistant at lower levels. Comparative genomic hybridization analysis showed the acquisition of resistance to fluconazole was accompanied by significant genomic modifications manifested as chromosomal duplications. These findings indicate that heteroresistance to fluconazole in *C. neoformans* and *C. gattii* is an intrinsic mechanism of the fungus which may contribute to fluconazole therapy failure in cryptococcosis.

57. Withdrawn

58. Differential roles of the ChiB chitinase in autolysis and cell death of *Aspergillus nidulans*

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An autolysis of fungal cells becomes a predominant phenomenon in the absence of the regulator of G protein signaling (RGS) FlbA in *Aspergillus nidulans*. The secreted endochitinase ChiB was shown to play a major role in autolysis. We investigate the roles of ChiB in fungal autolysis and cell death through genetic, biochemical and cellular analyses employing a set of critical mutants. Determination of mycelial mass revealed that under liquid-submerged culture conditions, while the *flbA* deletion (¥Ä*flbA*) mutant autolyzed completely after 3-d incubation, the ¥Ä*flbA* ¥Ä*chiB* double mutant escaped from hyphal disintegration. These results indicate that ChiB is necessary for the ¥Ä*flbA*-induced autolysis in submerged culture. However, importantly, both ¥Ä*flbA* and ¥Ä*flbA* ¥Ä*chiB* strains displayed dramatically reduced cell viability compared to wild type. These imply that ChiB is dispensable for cell death and that autolysis and cell death are separate and independent processes. LC-MS-MS analyses of the proteins that are progressively over- produced in the ¥Ä*flbA* and ¥Ä*flbA* ¥Ä*chiB* mutants identified downstream enzymes likely playing a key role in autolysis and/or cell death. We postulate a molecular mechanism bridging G protein signaling and autolysis.

59. A network of homeodomain transcription factors regulates growth and sexual development in *Podospora anserina*.

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Homeodomain (HD) proteins are DNA-binding proteins that play major roles in many developmental processes of animals, plants and fungi. Five homeobox genes were found in the complete *P. anserina* genome sequence. We investigated their function by deleting all of them and by generating all combinations of double mutants and the mutant deleted for all genes. Only 3 single mutants displayed phenotypes, either during vegetative growth as for the previously described *pah1* null mutant [1] or during sexual development. The mutants deleted for the gene homologous to *Neurospora crassa bek-1* and for *Hom2* developed aberrant perithecia without beaks and *Pa bek1* KO also affected maturation of ascospores. Interestingly, *Hom3* and *Hom4* null mutants, which did not harbor any phenotype, modulated *Hom2* KO phenotype. Additional genetic interactions were found between *pah1, Pa bek1* and *Hom2*. Overall, our data indicate that in *P. anserina* the HD transcription factors operate as a network to regulate growth and development. This work is funded by contract # ANR-05-BLAN-0385-01 from the ANR. 1-Arnaise *et al* Mol Microbiol. (2001) 39

60. The transcriptional program of *Podospora anserina* sexual development.

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A whole genome microarray approach has been engaged in the recently sequenced heterothallic euascomycete *Podospora anserina* (1) to identify genes that regulate the key steps of its sexual development. A time-course transcriptional study of 10 developmental stages from fertilization to ascospore maturation has been performed. More than half of the genome (58% of the 10540 CDS) displayed a significant change of transcript levels between at least two developmental stages of the kinetic (i.e. on all comparisons between the 10 developmental stages). Furthermore, to decipher the pathways specifically required during sexual development, a clustering analysis has been performed on a subset of selected genes: the gene expression program consists of temporal transcriptional waves that correlate with major morphological biological processes occurring during sexual differentiation. The overall data are currently under investigations. This project is funded by Agence Nationale de la Recherche, contract n° ANR-05-0385- 01. (1) Espagne E. *et al.*, Genome Biol, 2008. 9: R77

61. *Fusarium* comparative genomics: connectivity between genome innovation and pathogenicity.

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We will present the results of comparative genomics analysis among three economic important and closely related Fusarium species: *F. graminearum, F. verticillioides* and *F. oxysporum*. More than 90% of the *F. verticillioides* genome can be unambiguously aligned to the syntenic regions in *F.oxysporum* with an average 90% sequence identity. Specifically, all eleven chromosomes in *F. verticillioides* have corresponding chromosomes in *F. oxysporum and F. graminearum*. In contract, over 15 Mb sequences, including 4 of the 15 *F. oxysporum* chromosomes, lack significant orthologous sequence in the other two genomes, defining the *F. oxysporum* lineage specific regions (*Fol* LS regions). These *Fol* LS regions are enriched for secreted proteins, transcription factors, and genes involved in signaling transduction regulation. The secreted proteins encoded in the *Fol* LS regions include known virulence factors such as, *SIX* (Secreted in Xylem) proteins, Necrosis and ethylene-inducing proteins, Peroxidases, and plant/fungal cell wall degrading enzymes. Gene families important in lipid metabolism and generation of lipid-derived second messengers are also expanded through the gene encoded in these *Fol* LS regions. The evolutionary mechanisms underlying the acquisition and diversification of such genetic material and their potential impact on the pathogenicity will be discussed.

62. Genome sequence of the basidiomycete *Heterobasidion annosum*

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The genome sequence of the conifer rot root pathogen *Heterobasidion annosum* was generated at JGI with 8.23 X coverage. The nuclear genome assembles in 39 scaffolds of total 33.7 Mbp estimated to cover 98.1% of the complete genome. We predicted 12,270 genes with an average length of 1,617 bp and exon number and length of 5.54 and 250 bp respectively. About 50% (5999) of the predicted genes could be validated by EST support with the 40,807 EST´s generated with in the project. The genome has a GC content of 52.0% and very little repetitive sequences with 2,895 SSR per mega base. The physical genome is congruent with the genetic linkage map, and most of the linkage groups have been possible to anchor to the 18 largest scaffolds.

63. Systematic discovery of regulatory motifs in Fusarium by comparison of four Fusarium genomes.

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Comparative genomics provides a powerful tool for computational discovery of regulatory motifs in a set of closely related genomes. The evolutionary tree of the four Fusarium species, *F. graminearum*, *F. verticillioides*, *F. oxysporum* and *F. solani*, has a total branch length of ~1.1 substitutions per site (in noncoding regions), making it ideal for discovering regulatory motifs using comparative genomics. We developed a motif conservation z-score (MCS) to quantity the level of cross-species conservation for each candidate motif and a computational pipeline to identify motifs with high MCS to create a systematic catalogue of common regulatory motifs in promoters and 3'UTRs. The computational pipeline consists of enumerating candidate k-mers, calculating MCS for each of them, extending conserved k-mers to allow degeneracy, and finally merging extended k-mers to derive a set of distinct motifs. Using a high threshold score of MCS>10, we identified 22 candidate motifs in the promoters and 20 candidate motifs in the 3'UTRs. A number of motifs discovered in the promoters showed high similarity to regulatory motifs in yeast, such as CBF1, SKO1, PHO4, MBP1 and SWI6, suggesting a high conservation of transcriptional regulatory mechanism across different fungal clades. The genes associated with the identified motifs are enriched for certain function categories based on GO annotation. The biological significance of the motifs is also tested using the *F. graminearum* gene expression data.

64. A systematic analysis of T-DNA insertion patterns in the genome of *Leptosphaeria maculans*

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Agrobacterium tumefaciens-mediated transformation (ATMT) has been used for the transformation of more than seventy fungal species. ATMT offers numerous advantages over more conventional transformation methods and has led to the identification of genes involved in pathogenicity, pigmentation, conidial morphology and germination. However, data on the chromosomal distribution of the T-DNAs are scarce especially in fungi because, in most studies, only a few flanking sequences were rescued. As part of a large-scale insertional mutagenesis project aiming at the discovery of pathogenicity factors in the Dothideomycete fungus *Leptosphaeria maculans*, a collection of more than 5000 transformants has been generated and a set of 250 flanking sequences was obtained by thermal asymmetric interlaced-PCR and PCR-walking. The *L. maculans* whole genome sequence showed a very unusual genome structure for ascomycete fungi: the alternation of large GC-rich and AT-rich regions similar to isochores of higher eukaryotes. Genome wide plotting of T-DNA tags revealed a bias of insertions in favour of GC-rich isochores of larger chromosomes. Within the latter, integration events were significantly higher in intergenic sequences of gene-rich regions (60%). Besides, 81% of T-DNA's insertions in predicted genes occurred in exons. Further analysis of genes and genomic sequences surrounding the insertion sites will be presented.

65. Comparative Analysis of Peptidase Expression Profiles Between *Trichophyton tonsurans* (TT)and *Trichophyton equinum* (TT)
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Little is known about TT, the most common fungal pathogen of children. Notably, the organism shares a high degree of homology with the zoophilic TE. Comparative analyses between these spp. provide a unique opportunity to examine the genes that may select for humans vs. animals. TT (n=5) and TE (n=4) were inoculated in aqueous medium containing keratin as the sole nutrient. Isolates were harvested at 4, 7, 10, 12 and 14 d and total RNA isolated. The expression of selected peptidases (aminopeptidase P; carboxypeptidases M14, S1', Y; leucineaminopeptidase 1; dipeptidylpeptidases 4, 5) were evaluated by q-PCR. In both spp., CarbY, AmPP and Dpp4 showed the highest rate of expression at the earliest observable time point decreasing over the course of the study ($p<0.01$). In TT, Dpp5 and CarbS1' showed a similar expression profile to the above genes ($p<0.01$); however, there was no appreciable change in gene expression in TE. In contrast, Lap1 demonstrated a reduction in expression profile over the course of the study in TE ($p=0.09$) with no appreciable change observed in TT. Within gene families, DPP expression appeared superimposable with Dpp5 expression on average 7x greater than Dpp4. Among the carboxypeptidases, CarbY and CarbS1' had a comparable magnitude of expression while CarbM14 demonstrated negligible expression over the course of the study. The unique differences observed CarbS1', Lap1 and Dpp5 expression suggests that these genes may be involved in host selection.

66. Evolution of nonribosomal peptide synthetases: generating chemical diversity
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Non-ribosomal peptide synthetases (NRPSs) are multimodular enzymes that make non- ribosomal peptides (NRPs) through a thiotemplate mechanism independent of ribosomes. NRPs have important roles in both development and niche-specific success of filamentous fungi in addition to the well-known, useful, biological effects on other organisms including antibiotic, immunosuppressant, antitumor, and virulence-promoting activities. The modular structure of NRPSs, consisting of repeated units of Adenylation (A), Thiolation (T), and Condensation (C) domains, allows for both rapid evolution of novel genes, as well as flexibility in biosynthetic strategies. The mechanisms by which these genes evolve are likely complex, involving tandem duplication, duplication and loss, recombination, gene conversion, and fusion/fission of modular units (either single domains or A-T-C modules). We have addressed this issue, using several phylogenetic approaches in two datasets: 1) among homologs of the relatively conserved NRPSs that biosynthesize intracellular siderophores found in all filamentous ascomycetes and some basidiomycetes and 2) among NRPSs found in closely related Dothideomycete species. Our results suggest that 1) tandem duplication of complete A-T-C units represents the most plausible explanation for the generation of multimodular genes, 2) loss and/or swapping of A domains involved in substrate recognition may represent a mechanism for rapid evolution of new compounds, and 3) genes conserved across filamentous ascomycetes are also conserved in closely related species, while others appear prone to rapid duplication and rearrangement.

67. Evaluation of computer predictions in the genome of the ectomycorrhizal basidiomycete *Laccaria bicolor*
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In 2008, the sequenced genome of *Laccaria bicolor* was published (Martin et al. Nature 452:88). Some 19,000 genes were predicted in the genome by computer programs. Average lengths of introns and exons were calculated. Intron length was given in the paper with in average 92 bases. Having annotated as part of the *Laccaria* annotation consortium many genes of the fungus, this value did not correspond to the experienced observations on gene structures. Thus, 100 genes linked to the *A* mating type locus on a chromosome highly conserved to *Coprinopsis cinerea* were carefully checked for potential mistakes in their gene structure. A number of far too long introns needed corrections. Computer mistakes identified were an inability to identify a short first coding exon, an inability to identify very small exons of 3 to a few bases and wrong linkage of two different genes into one with a long predicted intron in between. More than 800 introns were analyzed: The typical intron is around 50-55 bp long and only three were longer than 75 bp. Thus, the published value of 92 bp suggests that there is still a large body of false predictions in the annotated genome. Restriction of intron length between values of 35 to 75 bp in computer analysis may help out. A further observation from this study is that exon length can vary from a few bp to sizes over 1000 bp and there is no preferred exon size. Mathematical games of calculating average exon sizes are thus of little meaning.

68. Comparative transcriptome analysis of *Cryptococcus neoformans* responding to differential expressions of carbonic anhydrase
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Carbon dioxide sensing plays a pivotal role in survival and proliferation of pathogenic microbes due to the drastic difference of CO2 levels. Carbonic anhydrases(CAs) are not only key CO2-metabolic enzymes catalyzing reversible interconversion between CO2 and bicarbonate, but also important CO2-signaling modulators. *Cryptococcus neoformans* contains two functional carbonic anhydrases, Can1 and Can2. Can2 is essential for growth in normal air conditions and sexual differentiation. Since the *can2* deletion mutant having growth defects under normal air conditions is not appropriate for this purpose, we constructed the strain in which *CAN2* expression can be controlled by replacing the native *CAN2* promoter with copper-regulated promoter. Compared to the wild-type H99 strain, the *CAN2* promoter replacement strain did not show any growth defects in YNB medium containing a copper chelator but exhibited severe growth defects in YNB medium containing a copper sulfate that represses the expression of the CAN2 gene. By employing comparative transcriptome analysis we will elucidate the downstream genes regulated by differential expressions of carbonic anhydrases in *C. neoformans*. This study will provide further insights into the signaling network of CA/CO2-sensing pathway in pathogenic fungi.

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69. Further characterization of temperature sensitive, unknown mutations in *Neurospora crassa.*

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In our continuing effort to add value to the materials in the FGSC collection, we have endeavored to characterize genetically mapped but otherwise unknown temperature-sensitive mutations in *Neurospora crassa.* Initial efforts have led to the identification of mutations in two alleles of *un-16* as occurring in highly conserved amino acids in a ribosomal S9 protein as well as the demonstration that the mutation in *un-4* occurs in a translocase of the inner mitochondrial membrane (*tim16*). More recently, we have leveraged information from early genome library work by Schmidhauser and colleagues to identify *un-10* as a missense mutation in eukaryotic translation initiation factor 3B. Finally, we are characterizing *un-7* and preliminary results indicate that it is defective in a small nucleolar ribonucleoprotein complex subunit that has significant similarity to the human cirhin gene responsible for adolescent onset cirrhosis of the liver in Cree Indians. Additional efforts include cross-species complementation and introducing specific temperature-sensitive mutations into other fungi.

70. The genome of the zygomycete *Phycomyces blakesleeanus,* a model organism for sensory biology

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The zygomycete Phycomyces blakesleeanus is a model microorganism for research on sensory biology. The giant fruiting bodies of Phycomyces change their speed and direction of growth in response to environmental signals (light, gravity, wind, touch, and the presence of nearby objects). Additionally, light regulates fruit body development and induces the synthesis of the beta-carotene. Phycomyces genome analysis is expected to further elucidate the signaling pathways underlying its photoregulation. To this end the genome was sequenced to 7.49X depth and assembled into 475 scaffolds totaling 56 Mbp, and 47847 ESTs were assembled from cDNAs of light and dark cultures. We combined into a single annotation pipeline a variety of gene modeling methods (homology-based, EST-based, and ab initio), and predicted 14792 protein-coding genes. The initial characterization of the Phycomyces protein set has uncovered the presence of multiple photoreceptor proteins and large families of proteins involved in signal transduction. In addition, several new sets of repetitive DNA, including transposable elements, have been described in the genome of Phycomyces. We expect that the Phycomyces genome will help to understand the molecular mechanisms for sensing of light, gravity and other environmental signals.

71. The genome of *Blumeria graminis*: massive size expansion in an obligate biotroph

The BluGen consortium, Pietro Spanu et al, www.BluGen.org

We have sequenced the genome of *Blumeria graminis f. sp. hordei* (DH14). About 10x coverage of the genome was achieved using Sanger, 454 and Illumina sequencing. The genome is estimated to be around 120Mb, which is about 3-4 times larger than related Ascomycetes. The increase is due to an abnormal expansion of transposable elements (>70% of the genome), most of which are novel and uncharacterised (See Amselem *et al.*, this meeting) whilst the size non-repetitive gene space is congruent with similar fungi. The repeats are highly interspersed and are the result of ongoing active retro-transposition. We are currently annotating the genome using *ab initio* and similarity (EST mining and proteogenomics) based gene prediction pipeline. Conventional cDNA sequencing of 6 different developmental stages and high- throughput sequencing of 5' cDNA ends from sporulating epiphytic hyphae has lead to the definition of over 7700 unique genes (out of predicted total of ~11000) and to the discovery of high heterogeneity at the start of transcription, including the frequent presence of non-template bases. Proteogenomics of conidia, epiphytic hyphae and infected barley epidermis identify experimentally over 600 proteins. This reveals changes in the *Blumeria* proteome during development and disease, including a large complement of secreted proteins produced specifically by haustoria. It is expected that completion of the annotation including manual curation of the data will provide unique insights in the evolution, development and biology of powdery mildews and obligate biotrophy.

72. Genome sequencing of *Aspergillus awamori* and comparison with the *Aspergillus oryzae* genome

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Aspergillus awamori is widely used for brewing Japanese traditional spirits, awamori, in Okinawa prefecture. High potential of secretory enzymes and the safety of *A. awamori*, makes this microorganism important also for modern biotechnology. *A. awamori* is genetically very close to *Aspergillus niger* and close to *Aspergillus oryzae*. Like *A. niger*, *A. awamori* vigorously produces citric acid. We have sequenced *Aspergillus awamori* NBRC 4314 (RIB 2604). The total length of non-redundant sequences reached 34.7 Mb comprised of contigs fallen into 44 major linkage groups. Comparison between the genomes of *A. awamori* and *A. oryzae* showed higher diversity of genes located on the non-syntenic blocks of the *A. oryzae* genome. Considering close relationship between the two species industrially used in a similar way, detailed comparison between the two genomes including other Aspergillus species will provide useful information to elucidate gene function of the two species.

73. New chronotype mutants in Neurospora discovered through entrainment

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Neurospora is one of the simplest genetic models for the circadian clock. Using traditional methods (mutant screens and mapping) together with monitoring circadian regulation of spore formation, a negative transcriptional feedback loop has been described as central to the circadian clock mechanism. However, many lines of evidence suggest that there are additional clock genes that remain to be discovered in this fungus. We used the annotation of the Neurospora genome to find candidate clock genes, namely proteins coding for putative photoreceptors, some of which contain PAS domains, (a motif common to all eukaryotic clock gene networks). We obtained knockout mutants and screened them in a variety of protocols for circadian rhythm and entrainment. We find that several of the photoreceptor mutants show alterations in their entrained phase. The same strains show a normal circadian rhythm (free running period). These chronotype mutants are thus exceptions to the principle concerning the relationship between period and phase. David lenssen Dept. of Chronobiology Biological Centre University of Groningen Postbus 14 9750AA Haren The Netherlands D.M.J.Lenssen@rug.nl

74. Secondary metabolite engineering in the fungus *Fusarium verticillioides*.

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The filamentous fungus *Fusarium verticillioides* (synonym: *F. moniliforme*, teleomorph: *Gibberella moniliformis*) is a pathogen of corn (*Zea mays*), causing kernel, stalk and ear rot; it is one of the most common fungi associated with corn intended for human and animal consumption. A large multidomain enzyme, Fusarin Synthase (FUSS) has been identified from the *F. verticillioides* genome that is involved in the biosynthesis of the toxin Fusarin C. This enzyme is especially interesting as it is encoded by a gene (*fusA*) that has a fused polyketide synthase – non ribosomal peptide synthetase (PKS-NRPS) structure, one of the first to be discovered in fungi.

Swapping of individual domains has been attempted in fungal PKS genes, with some success, however this project involves swapping whole NRPS modules to make novel chimaeric PKS-NRPS enzymes, using the *fusA* PKS as a backbone. The first chimaeric PKS-NRPS to be constructed used the NRPS module from the gene *tenS*, another PKS-NRPS from the insect pathogen *Beauveria bassiana. tenS* encodes the enzyme Tenellin Synthase which is involved in the early synthesis steps of the 2-pyridone, Tenellin. This swap has been performed using homologous recombination (HR) in *Saccharomyces cerevisiae*, which is a particularly flexible technology for working with large genes where convenient unique restriction sites are often not present. Expression of the chimaeric PKS-NRPS gene(s) will be tested in both *F. verticillioides* itself and a heterologous host, the filamentous fungus *Aspergillus oryzae*.

The biosynthetic pathway for the production of Fusarin C is also being investigated. FUSS itself does not contain enough catalytic functions to synthesise Fusarin C, and some predictions have been made about the structure of 'pre-Fusarin C' using sequence analysis and evidence from the structure of Fusarin C. It is likely that pre-Fusarin C would consist of a tetramethylated heptaketide with a homoserine moiety. Four further post-PKS modifications would be required to produce mature Fusarin C: epoxidation, oxidation of a pendant methyl to a carboxylate and esterification, and hydroxylation α to the nitrogen. Genes clustered around *fusA* in the genome are likely candidates for post-PKS processing, and several genes have been identified for both knockout (KO) and gene silencing.

75. Characterization of the MAT1-1 mating-type locus of the asexual cephalosporin C producer *Acremonium chrysogenum*

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Acremonium chrysogenum, the fungal producer of the beta-lactam antibiotic cephalosporin C, is classified as asexual because no direct observation of mating or meiosis has yet been reported. To assess the potential of sexual reproduction in *A. chrysogenum*, we screened an expressed sequence tag (EST) library of *A. chrysogenum* for the expression of mating-type (MAT) genes. We identified two putative mating-type genes that are homologues of the alpha-box domain gene MAT1-1-1 and MAT1-1-2 encoding a HPG domain protein. The entire AcMAT1-1 and regions flanking the MAT region were obtained from a genomic cosmid library, and sequence analysis revealed that in addition to AcMAT1-1-1 and AcMAT1-1-2, the AcMAT1-1 locus comprises a third mating-type gene AcMAT1-1-3 encoding an HMG-domain protein. To determine the functionality of the AcMAT1-1 locus, the entire MAT locus was transferred into a MAT deletion strain of the heterothallic ascomycete *Podospora anserina*. After fertilization with a PaMAT1-2 (MAT+) strain, the corresponding transformants developed fruiting bodies with mature ascospores. Thus, the results of our functional analysis of the AcMAT1-1 locus provide strong evidence to hypothesize a sexual cycle in *A. chrysogenum*.

76. T-DNA insertion mutagenesis to identify sclerotial development genes in *Sclerotinia sclerotiorum*.

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Sclerotinia diseases cause over $100M loss to vegetable crops annually in Australia, with significant losses worldwide. Control of these diseases is severely impeded by the pathogen's ability to produce sclerotia; highly melanized structures crucial to the pathogen's propagation, reproduction and survival. T-DNA mutants have been created via *Agrobacterium*-mediated transformation. Approximately 1200 T- DNA mutants were created and screened for altered sclerotium phenotypes. Approximately 40 of these present a sclerotia-minus, or aberrant sclerotia phenotype and are currently being analyzed. Various methods such as TAIL-PCR and Genome Walking are being utilized in an attempt to identify the insertion point of the T-DNA. Other phenotypic characteristics have also been screened including pathogenicity and acid production. Two sclerotia-minus mutants with varying levels of acid production, compared to wild type, have been identified. It is hoped that any genes identified as contributing to sclerotial formation may provide potential targets for long-term, sustainable control of *S. sclerotiorum* and related soil-borne pathogens.

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77. Elimination of selectable marker from various filamentous fungi by transient expression of Cre recombinase

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A convenient method to reuse a selectable marker would be advantageous for studies requiring sequential transformations of fungi. For example, only the hygromycin B- resistance marker (*hph*) has proven useful for most *Epichloë* and *Neotyphodium* species. Furthermore, due to their heteroploid nature several *Neotyphodium* species tend to have multiple gene copies, necessitating sequential disruption for functional genetic analysis. Also, removal of markers should reduce regulatory impediments to studies or applications that involve environmental release of genetically modified fungi. We implemented an approach to remove selectable markers, which requires only that the markers be flanked by loxP sites in direct orientation ('floxed'). Protoplasts from *Epichloe festucae, Neotyphodium coenophialum* or *N. uncinatum* transformants containing floxed *hph* were treated with a fungus-active *cre* gene, then cultured without selection. The marker was eliminated in 0.5-2% of the colonies, leaving a single loxP sequence. The *cre* gene was not integrated into the genome. Similar results were obtained with a floxed *yA* marker in *Aspergillus nidulans*. Thus, this approach is rapid, efficient and useful for diverse filamentous fungi.

78. Using the Pathogen-Host Interactions database (PHI-base) and ONDEX data integration platform to enhance comparative genomics analyses

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The PHI-base database (www.PHI-base.org/) is a unique multi-species pathogen resource because it only contains expertly curated molecular and biological information on genes proven to affect the outcome of pathogen-host interactions. This information is retrieved from the peer reviewed scientific literature. Since 2007 it also includes information on known fungicide target sites. PHI-base currently contains 1185 entries (n = 950 genes / 1185 interactions) for experimentally verified pathogenicity, virulence or effector genes from plant, fungal, insect and animal attacking fungi, Oomycetes and bacteria.

ONDEX combines comparative genomics with data integration and can be used to predict potential pathogenicity, virulence and effector genes in any species.

By combining the annotated PHI-base entries with ONDEX we have explored the entire contents of PHI-base as well as the gene repertoire predicted to be required for the disease causing ability of the globally important cereal attacking pathogen *Fusarium graminearum (Fg)* . This has revealed which predicted proteins represent ancient conserved pathogenicity components, which are plant or animal pathogen specific and those which are single species specific.

79. Genomic and functional analysis of three *argonaute* genes in *Mucor circinelloides*.

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Argonaute proteins are the effector components of the RNA silencing machinery, causing the endonucleolitic degradation of the complementary mRNA to the antisense strand of the siRNAs. By using degenerated oligonucleotides we have cloned three different *argonaute* (*ago*) genes in the zygomycete *Mucor circinelloides* and have analyzed the function of these genes in transgene-induced gene silencing. Null *ago-1* mutants are severely impaired in RNA silencing induced by sense and inverted repeat transgenes, suggesting a primary role of this gene in the silencing mechanism. It is also involved in the control of endogenous functions, since sporulation efficiency in this mutant is greatly reduced compared with the wild type. The latter phenotype is also shown by null *ago-2* mutants, even though this gene contains a premature stop codon, which would produce a truncated protein lacking the PIWI domain. The presence of all the conserved residues of the PAZ and PIWI domains in Ago-2, together with the effect of the null mutation suggest that a ribosome frame shift might occur during Ago-2 translation. No phenotype has been identified for the null *ago-3* mutants, the gene being expressed at a very low level during the vegetative growth. Comparative genomic analysis of the zygomycete *ago* genes reveals a certain degree of synteny and suggests that genomic rearrangement have occurred during zygomycete evolution.

80. Impact of changes in the target P450 CYP51 enzyme associated with altered triazole- sensitivity in the cereal crop pathogen *Mycosphaerella graminicola*

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The triazoles are a widely used class of fungicides, targeting the cytochrome P450 sterol 14alpha-demethylase *Cyp51*. Despite heavy use of this chemical class in the field over a considerable period of time, catastrophic resistance has not been observed. Rather, there has been a slow shift toward reduced sensitivity. A number of mutations in the *Cyp51* gene have been previously associated with this shift, although other resistance mechanisms such as alteration in sterol biosynthesis and fungicide uptake and efflux may also play a role. There have been attempts to correlate changes in resistance levels with specific *Cyp51* mutations in field isolates. However, due to the genetic diversity of *Mycosphaerella* and the possible effect of non-target site mutations, making solid conclusions has been problematic. We have introduced a number of mutations into the *Cyp51* gene of the economically important plant pathogen *Mycosphaerella graminicola*, with the aim of determining the contribution each substitution has on triazole efficacy, in a uniform genetic background. Here, we present our findings of the comparative efficacy of varying triazole structures against our range of mutants.

81. The *Aspergillus carbonarius* genome: Analysis of potential secondary metabolite biosynthetic gene clusters.

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A draft genome sequence for *Aspergillus carbonarius* strain ITEM5010 has been recently generated in collaboration with the US Department of Energy Joint Genome Institute. *Aspergillus carbonarius* is closely related to *Aspergillus niger*, an important model organism as well as an industrial enzyme and organic acid producer. Like *A. niger*, the genome sequence for *A. carbonarius* encodes numerous glycoside hydrolases. In addition, the *A. carbonarius* genome sequence will aid in annotation of *A. niger*. *Aspergillus carbonarius* is also an economically significant organism as the main agent of ochratoxin A contamination of wine. We have characterized the putative ochratoxin A biosynthetic cluster and have initiated manual annotation of the draft genome sequence. While *A. niger* has been recently shown to produce fumonisin B2, the biosynthetic cluster for fumonisin biosynthesis is absent from the *A. carbonarius* genome. We have initiated a detailed analysis of the polyketide synthase (PKS) genes encoded within the *A. carbonarius* genome. Preliminary phylogenetic analysis of ketosynthase domains indicates a substantial diversity within predicted PKS encoding genes.

82. Screening for new meiotic genes in *Coprinopsis cinerea* (*Coprinus cinereus*)

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The basidiomycete *Coprinopsis cinerea* (*Coprinus cinereus*) exhibits synchronous development of its meiotic tissues, making it ideal for the study of this developmental process. We have recently exploited this aspect of the mushroom's development to describe meiosis using a full transcriptome microarray, which provided many exciting candidates for important meiotic genes. Screening for meiotic defects in *C. cinerea* is facilitated by the absence or reduction of spores produced, resulting in a white or gray appearance of the gill tissue, as opposed to the usual black. We are currently developing methods to exploit this easily-defined phenotype, in concert with high-throughput RNAi, to identify meiotic genes of interest, and progress will be reported.

83. Small RNA Pathways in the Oomycetes *Phytophthora sojae*, *Phytophthora ramorum*, and *Phytophthora infestans*.

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The oomycetes are a distinct lineage of eukaryotes that contain many important pathogens of plants, including *Hyaloperonospora parasitica* and several *Phytophthora* species. Plant pathogens of the genus *Phytophthora* are among the most well-studied oomycetes and include *P. infestans*, a pathogen of potato that caused the Irish potato famine, *P. sojae*, a pathogen of soybean, *P. ramorum*, the cause of sudden oak death, and *P. capsici*, a pathogen of pepper. Recently the genomes of these four *Phytophthora* species and *H. parasitica* were sequenced. Most eukaryotes have RNA silencing systems that use small RNAs to suppress a wide range of genes, genetic elements, and viruses. One important silencing pathway is the microRNA pathway. miRNAs (usually 21-22 nucleotides) are derived from processing of self-complementary foldback RNAs derived from *MIRNA* genes. miRNAs associate with protein effector complexes containing ARGONAUTE proteins, and the miRNA serves to guide cleavage, translational repression or redirection of the target transcript within the cell. Here, we identified the core small RNA biogenesis components and effectors in three *Phytophthora* species, *P. sojae*, *P. ramorum*, and *P. infestans*. We used high-throughput pyrosequencing (454 Life Sciences) and sequencing-by-synthesis (Illumina) to profile small RNA. Analysis of these data revealed several candidate *MIRNA* genes from one gene family that are conserved in all three species. In addition, large numbers of siRNA-generating loci were identified throughout the *Phytophthora* genomes.

84. Microarray identification of genes differentially transcribed in strains of opposite mating types in *Podospora anserina*.

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Fertilization in the heterothallic *Podospora anserina* is controlled by the expression of the MAT1-1-1/FMR1 and MAT1-2-1/FPR1 transcriptional regulators in *MAT1-1/mat-* and *MAT1-2/mat+* partners, respectively. FMR1 and FPR1 candidate target genes were identified by comparing the transcriptomic profile of four strains: sexually proficient wild-type *mat-* and *mat+* strains, and *fmr1* and *fpr1* loss-of-function mutants. Differentially transcribed genes because of DNA polymorphism around the idiomorphs were eliminated from the analysis. One class of differentially transcribed genes consists of well-known mating-type target genes, as the pheromone receptor genes, the pheromone genes and the genes required for the pheromone processing. Another class contains numerous genes with no known function in fertilization. Their possible function during sexual cycle is currently investigated by deletion. These two classes amount to a total of 113 differentially transcribed genes that are controlled by activation and/or repression by either FMR1 or FPR1, or both of them, suggesting a very complex pattern of regulation. This work is funded by contract # ANR- 05-BLAN-0385-01 from the ANR.

85. The transposome of *Cryphonectria parasitica*.

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Mobile genetic elements such as transposons (TEs) are DNA sequences capable of inserting into chromosomes and proliferating to high numbers within the host genome. Because of these traits, TEs influence the evolution of eukaryotes through modifications such as altered genome size, disrupted or enhanced gene expression, and disordered chromosomal organization. Despite the interactions between TEs and their hosts, comprehensive genome-scale studies of these elements in the filamentous fungi have not been reported. Here we describe the entire complement of TEs populating the genome of *Cryphonectria parasitica* strain EP155, the first transposome characterized for any filamentous fungus, and provide an overview of the evolution of these elements. Few intact TE copies survive, and several lineages are extinct, but full- length pseudogenes are maintained for all classes. Unlike all other ascomycetes surveyed, there is no evidence of repeat-induced point mutation (RIP) in the EP155 genome, despite the observation that the majority of pseudogene mutations were caused by nucleotide transitions. Even without RIP silencing, TEs do not overpopulate EP155, with ~10% of the 43.9 Mb genome comprised of TEs, consistent with reports from related fungi. The distribution of TEs is highly skewed. Retrotransposons of the Metaviridae family are abundant (~9% of total genomic DNA), but appear to survive only as pseudogenes, clustered in large groups. In contrast, a small portion of the genome is derived from other TE families, with all other TEs, combined, comprising only 0.81% of the EP155 genome. In particular, only 0.34% of the genome is comprised of type II DNA transposons, typically the most abundant family of TEs observed in filamentous fungi.

86. RIP and recombination in the commercially used fungi *Aspergillus niger* and *Penicillium chrysogenum*

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We have analyzed the transposon content in the genomes of *A. niger* and *P. chryosogenum* (1). In both fungi there is strong evidence for a repeat induced point mutation (RIP)-like mechanism (2), which was found in multiple copies of the *A. niger* transposon *AniTa1* and the *P. chrysogenum* transposon *PeTra2*. For *PeTra2* all sequences investigated seem to be effected by RIP in a varying extend. *AniTa1* copies seem to be unRIPed with the exception of two copies in which 20% of all nucleotides have been altered due to RIP. We have identified a recombination event between two copies of the retrotransposon *ANiTa1* within the genome of the fungus *A. niger* CBS513.88. The observed chromosomal rearrangement appears to be strain-specific. The striking genomic differences in *ANiTa1* copy distribution leading to differences in the chromosomal structure between the two strains, ATTC1015 and CBS513.88, suggest that the activity of transposons may profoundly affect the evolution of different fungal strains (3).

1. Fungal Genet Biol(2007) 44:1399-1414 2. Curr Genet (2008) 53:287-297 3. Mol Gen Genom (2008) 280:319-325

87. Fungal transposons: From mobile elements towards molecular tools

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Transposons are mobile and mostly also repetitive sequences, which are found in all eukaryotic genomes. We have analyzed the transposon content in two fungal genomes, *A. niger* and *P. chryosogenum*. One non-autonomous element, the *A. niger* transposon *Vader*, was shown to be active during strain development (1). *Vader* mobility could also be shown in a transposon trap experiment. Due to its obvious activity and to its ability to insert into genes *Vader* appears to be suitable as a gene tagging tool. We present evidence for excision of *Vader* from a vector sequence. Finally we show data regarding the fungal transposon *Restless* (2, 3) and its use as a molecular tool. To this end we have expressed the *Restless* transposase gene in *N. crassa*.

1. Fungal Genet Biol (2007) 44:1399-1414 2. Mol Cell Biol (1996) 16:6563-6572 3. Mol Gen Genet (2000) 263:302-308

88. Identification of repetitive elements in the *Coprinus cinereus* genome

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Repetitive DNA sequences are a feature of many eukaryotic genomes and range from simple repeated sequences to highly complex structures. While the function of repetitive sequences is not well understood, it appears these elements play important roles in chromosome function and stability. A study was conducted to characterize the DNA repetitive elements within the genome of the basidiomycete *Coprinus cinereus* using the sequence data generated by the Broad Institute Fungal Genome Initiative. An analysis of the genome sequence using PILER developed a library of 29 repeat families. The copy number and distribution of these families was explored in the *C. cinereus* genome using RepeatMasker. A BLASTX analysis of the 29 families showed five families with strong hits to retrotransposons (Class I elements). Further analysis of those five families identified four as gypsy (Ty3)-like and one as copia (Ty1)- like elements. Retrotransposon-like elements make up approximately 2.2 % of the *C. cinereus* genome. To identify possible *C. cinereus* transposons (Class II elements), a search of annotated Pfam domains and gene predictions identified 26 sequences with similarity to Class II elements in the genome. These elements represent three types of transposable elements: Activator, Enhancer and Mariner.

89. Transcriptome landscapes of *Coprinopsis cinerea* dikaryotic mycelium and primordium

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Coprinopsis cinerea is a model mushroom to study Basidiomycete fruiting body development. To identify essential genes during the process, we profiled polyA+ transcriptome of two important developmental stages - dikaryotic mycelium and primordium - using high-throughput 5'SAGE and microarray analysis. For 5'SAGE, 116,000 mycelium tags and 157,000 primordium tags were obtained from a Roche 454 GS20 run. High-quality tags were mapped to genome sequence. Tags upstream to protein-coding gene models were counted to assign gene expression levels and transcriptional start sites. Mycelium and primordium RNA were also profiled using quadruplicate of 13,230-oligo array. Normalized expression ratios of every oligo between two stages were then integrated with 5'SAGE data using MySQL database. Significant differentially expressed genes based on both or either platform were categorized according to their annotations, including ortholog, protein domain and Gene Ontology. Differential expressions of selected subsets of these genes were confirmed by quantitative real-time PCR. All the integrated data in database are available through a user-friendly website.

90. The *Pleurotus ostreatus* laccase multi-gene family

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A laccase gene family of seven members has been characterized in the basidiomycete fungus *Pleurotus ostreatus* and their closely related spatial organization on the fungal genome has been demonstrated. All the *P. ostreatus* laccase genes, *pox1*, *poxc*, *poxa1b*, *poxa3*, *pox3*, *pox4* and *pox5* proved to be located within a 150 kb genomic region. *pox3* and *pox5* are situated at a 800 bp distance, *pox1* is far no more than 10 kb from *poxa3* and *poxa1b*. Mature transcripts were isolated coding for all the laccase genes, except for *pox5*, for which no RNA could be detected in anyone of the tested conditions. Comparison of laccase gene structures revealed that *pox4* shows exactly the same gene organization of *pox1* and *poxc* genes. On the other hand, *pox3* and *poxa3* exhibit very different structures from that of the other members. *pox3* is characterized by a shorter length, related to a reduced number of introns. The high sequence similarity and the identical intron-exon structure shared by *poxc*, *pox1* and *pox4* genes along with the high degree of identity among the corresponding protein sequences indicate a close relationship between these genes and suggest the existence of a "laccase subfamily" consisting of these members.

91. SMURF: genomic mapping of fungal secondary metabolite pathways

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Filamentous fungi produce an impressive array of secondary metabolites (SMs) including mycotoxins, pigments, antibiotics and pharmaceuticals. The genes responsible for SM biosynthesis, export, and transcriptional regulation are often found in SM gene clusters. We developed the web-based software SMURF (Secondary Metabolite Unknown Regions Finder) available at www.jcvi.org/smurf/ to search systematically for such clusters. SMURF's predictions are based on the domain content of genes located close to backbone genes that often encode the first steps in SM pathways. In tests, SMURF accurately recovered all known SM clusters and detected 8 additional potential clusters in *Aspergillus fumigatus*.
We applied SMURF to catalog putative SM clusters in 25 publicly available fungal genomes. Among these taxa, the Aspergilli appear to have the coding capacity for the largest numbers of these metabolites. The genome of *A. niger* alone encodes 61 backbone enzymes and 58 clusters. Further comparison reveals that SM pathways are very unevenly distributed among fungal taxa suggesting an evolutionary pattern of rapid pathway gain and loss. It also confirmed the correlation between unicellularity and the absence of SMs, and the prominent role of gene duplication in the creation of new pathways.

92. Gene targeting in *Chrysosporium lucknowense* using a retrievable mutation in the KU70 gene.

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Current approaches for the development of fungal industrial host strains have long time- lines and the resulting strains are genetically ill-defined. Moreover, available approaches to resolve the latter bottleneck with targeted gene replacement have even longer timelines. A new approach based on a KU70 mutant strain resulting in a non- homologous recombination deficient mutant strain is reported for several non-industrial host strains. We used the KU70 approach for actual commercial strains of *Chrysosporium lucknowense*. For industrial applications the presence of a KU70 mutation in a final production host strain is unwanted as the recombination deficient KU70 phenotype may have adverse effects in large scale fermentation. Therefore, we designed two different KU70 disruption vectors. One type of KU70 disruption vector was designed for strain development approaches, involving multiple gene disruptions at different loci. In the primary KU70::*amdS* disruption strain the amdS selection marker can easily be removed by fluoroacetamide selection leaving a KU70 mutant phenotype. The subsequent gene disruption could be carried out using a reusable amdS selection marker. The other KU70 disruption vector was designed to disrupt the KU70 gene in a strain background to be used for construction of genetically defined production strains, by introducing a desired expression cassette e.g. at the *cbh1* locus. The wild type KU70 gene can easily be regenerated by fluroacetamide selection. The latter type of KU70 disruption vector can also be used to regenerate the WT KU70 locus in a multiple disrupted KU70 mutant host strain. The integration properties of the *Chrysosporium lucknowense* KU70 mutant strains will be presented.

93. Common, yet, distinct roles for two trichothecene regulatory genes in the phytopathogen, *F. graminearum*

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The two regulatory genes, *Tri6* and *Tri10* positively regulate the trichothecene pathway in *F. graminearum*. Here we report the phenotypic and transcriptomic characterization of both *tri6* and *tri10* mutants under stressed and non-stressed conditions. Targeted disruption of both of these genes failed to in vitro synthesize 15-acytyldeoxynivalenol, a derivative of the mycotoxin deoxynivalenol (DON). Further, infection on a Fusarium susceptible variety of wheat was restricted to the inoculated site. Massively parallel signature sequencing and microarray analyses revealed that *Tri6* negatively regulated "dispensable" gene clusters. These clusters are not required for normal growth of the fungus, but are activated only when the fungus is under stress. *Tri10* regulated only the subset of these dispensable clusters, but it also plays another role in the regulation of another mycotoxin pathway. Results suggest that *Tri6* can act as a positive and a negative regulator of gene expression. Consequences of multiple roles of these genes will be discussed.

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94. Intracellular pathogensis of filamentous fungi by the biocontrol bacterium *Lysobacter enzymogenes*.

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The gram negative bacterium *Lysobacter enzymogenes* infects a wide range of lower eukaryotes including filamentous fungi via internalization. We have been studying the molecular basis of *L. enzymogenes* with the filamentous ascomycetes *Cryphonectria parasitica* and *Magnaporthe oryzae*. The genome sequence of *L. enzymogenes* strain C3 is 6.1 Mb with a GC content of 69.5%. Initial global transcription and proteomic analysis have identified candidate genes regulated during the host-pathogen interaction. Further, *L. enzymogenes* appears to have at least one copy of every bacterial secretion system characterized to date. Bacterial secretion systems are intricate translocation machines that are often associated with pathogenicity and virulence of plants and animals. Using directed mutagenesis, we have been investigating the roles of Type 3, Type 4, and now Type 6 secretion systems on pathogenesis. *L. enzymogenes* appears to have two T6SS which contain the hallmark proteins present in all T6SS. To assess the roles of the T6SS pathways on lower eukaryotes, single and double deletion mutants of strain C3 lacking the Hcp proteins in each T6SS pathway are currently being constructed and the effect of the mutants on fungal infection will be monitored microscopically.

95. Phosphoproteome analysis and the role of protein kinases during sporulation in *Phytophthora infestans*.

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The asexual sporangia of *P. infestans* and the zoospores that they release serve as the primary inoculum for potato and tomato late blight. Expression-profiling studies using microarrays indicated that many protein kinases, as well as protein phosphatases, are differentially expressed during sporulation and zoosporogenesis; of these many are spore-specific. The role of protein phosphorylation in sporangial development is being addressed through two approaches. The first involves gene silencing of five spore- specific protein kinases, either using stable transformants expressing hairpin constructs and or by dsRNA-mediated transient silencing. The second entails a global analysis of phosphorylated proteins during sporulation. Proteins from non-sporulating mycelia, sporulating mycelia, and sporangia have been analyzed by two-dimensional electrophoresis. Phosphorylated proteins are detected using Pro-Q Diamond and total proteins using Sypro Ruby. Comparisons are then made to identify phosphoproteins that are newly synthesized during each developmental stage as well as proteins that have been modified by kinases or phosphatases. Excised spots are then subjected to mass spectroscopy and compared to *Phytophthora* sequence databases.

96. The putative vacuolar protein sorting 51 and 53 are required for osmotic-stress response and virulence in *C. albicans*

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C. albicans VPS51 was identified by microarray analysis as being up-regulated when *C. albicans* was in contact with endothelial cells. In *Saccharomyces cerevisiae*, Vps51p is known to form a complex with Vps52p, Vps53p, and Vps54p, which is required for protein sorting in the late golgi. To investigate the function of this complex in *C. albicans*, we constructed *vps51* delta / delta and *vps53* delta / delta null mutant strains, as well as *vps51* delta / delta ::*VPS51* and *vps53* delta / delta ::V*PS53* complemented strains. The *vps51* delta / delta and *vps53* delta / delta mutants had significantly reduced capacity to invade and damage both oral epithelial and endothelial cells in vitro. Also, these mutants were hypersusceptible to multiple stressors including congo red, glycerol, NaCl, SDS, hydrogen peroxide, protamine, Mn^{++}, and Cu^{++}. These mutants had profoundly attenuated virulence in murine models of oropharyngeal and disseminated candidiasis. The oral fungal burden of mice infected orally with the *vps51* delta / delta and *vps53* delta / delta mutants was at least 1,200-fold lower than mice infected with the wild- type strain. Also, none of the mice infected intravenously with either the *vps51* delta / delta and *vps53* delta / delta died, whereas mice infected with the wild-type strain had a median survival of 4 days. Interestingly, while mice infected with both mutants had 30-fold fewer organisms in their kidneys, they had 80-fold greater organisms in their brains, compared to mice infected with the wild-type strain. Therefore, Vps51 and Vps53 are required for *C. albicans* stress tolerance and virulence. They also influence the capacity of *C. albicans* to persist in specific organs during disseminated candidiasis. *Presenting author(lyaoping@labiomed.org)

97. The Neurospora crassa photoreceptors WHITE COLLAR-1, WHITE COLLAR-2 and VIVID impact carbohydrate metabolism in different ways

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Recently a connection between light response and cellulase gene expression was shown in Trichoderma reesei. Analysis of this effect in mutants of Neurospora crassa should show whether this is a unique phenomenon for this fungus or a conserved mechanism. In this study, we analyzed transcriptional profiles of wild-type and wc-1-, wc-2- and vvd- deletion mutants after 28 or 40 hours of growth on cellulose. We found that in all three photoreceptor mutants, cellulase activity is significantly enhanced as compared to the wild-type. Functional category analysis of statistically enriched transcripts in the set of significantly differentially regulated genes in the mutants revealed specific regulation of metabolic genes, especially of those involved in carbohydrate metabolism and amino acid metabolism. Interestingly, the transcriptional profiles of the vvd-mutant differ in many cases from those of the white collar mutants, although the output in terms of cellulase gene expression is similar. Our data point at a different set of hydrolytic enzymes and auxiliary proteins expressed in the mutants, acting synergistically to achieve higher efficiency, especially in the white collar mutants. We conclude that the connection between light signaling and carbohydrate utilization is a conserved mechanism in these fungi.

98. Novel gene discovery and molecular markers development of shiitake mushroom *Lentinula edodes* by high-throughput sequencing.
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Shiitake mushroom *Lentinula edodes* is a popular edible mushroom of high economic values. Next generation sequencing and bioinformatics tools were employed for the genomic analysis of *L. edodes* to reveal novel genes and to develop molecular markers for strains identification. A genome survey sequencing (GSS) of dikaryotic *L. edodes* L54 were performed using 454 GS-20 (Roche). A total of 353,030 reads (total length 35Mbp) were assembled into 31,000 contigs. To identify novel genes, the GSS data, the 12,000 unpublished in-house cDNA contigs and the 12,210 publicly available expressed sequence tag (EST) sequences in NCBI dbEST were searched using BLASTX against five other sequenced basidiomycetes. To develop simple sequence repeat (SSR) markers, 31,000 GSS contigs and the existing 24,210 EST sequences were used to search for SSR motifs, resulting in 587 sequences, containing at least 1 SSR motifs per sequence. A number of these SSR motifs were confirmed by cloning and sequencing. Their degrees of polymorphism across strains are being examined. To construct a draft genome sequence of *L. edodes*, as well as revealing more genes and SSRs, whole genome sequencing of monokaryotic strain L54- A, by both paired-end and shotgun approaches is ongoing using 454 GS-FLX (Roche).

99. Analysis of large chromosomal deletion construction and the roles of recombination- related genes in the *koji*-mold *Aspergillus oryzae*.
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Previously, we established techniques for efficiently constructing large chromosomal deletions in the *koji*-molds *Aspergillus oryzae* and *A. sojae*. In the present study, we have investigated the effect of the position of the targeted region of chromosomes on the efficiency of constructing large deletions in *A. oryzae*, and the roles of *ku70*, *ligD*, and *rad52* in the construction of large deletions. The efficiency of constructing large deletions in the near-telomeric region was compared with that in near-centromeric region. In the *delta ku70* strain, large deletions created via a loop-out recombination were efficiently obtained in the near-telomeric region but barely obtained in the near-centromeric region. In contrast, in both the *delta ku70* and *delta ligD* strains, large deletions were efficiently obtained by replacement-type recombination in the near-centromeric region. In addition, DNA double strand breaks induced by I-SceI endonuclease facilitated the construction of large chromosomal deletions in the near-centromeric region. Moreover, in the *delta lig4* and *delta ku70- rad52* strains, unintended deletions were created in the near-teromeric region by illegitimate loop-out recombination. These results indicated that the loop-out recombination is more sensitive to chromosomal structure than the replacement-type recombination, and that *ligD* and *rad52* play a role in the construction of large chromosomal deletions via a precise loop-out type recombination.

100. Analysis of the *Postia placenta* genome reveals a novel paradigm for lignocellulose depolymerization.
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In contrast to white-rot fungi which degrade both lignin and polysaccharides in wood, brown-rot fungi hydrolyze only cellulose and hemicellulose, leaving behind a modified lignin residue. While the economic consequences of brown-rot wood decay are substantial, detailed knowledge of the enzymatic and genetic machinery of these organisms lags behind that of white rot fungi. The genome sequence of *Postia placenta*, a brown-rot basidiomycete fungus, was recently completed by the Joint Genome Institute and an international consortium of co-investigators. Examinations of the *P. placenta* genome, transcriptome, and secretome have revealed a unique constellation of extracellular enzymes, including an unusual repertoire of extracellular glycoside hydrolases. Genes encoding exocellobiohydrolases and cellulose-binding domains, typical of cellulolytic microbes, are absent in this fungus. When *P. placenta* was grown in medium containing cellulose as sole carbon source, transcripts corresponding to several hemicellulases and to a single putative beta-1-4 endoglucanase were expressed at high levels relative to glucose-grown cultures. Also upregulated during growth in cellulose medium were putative iron reductases, quinone reductase, and structurally divergent oxidases potentially involved in extracellular generation of Fe(II) and H_2O_2. These observations are consistent with a biodegradative role for Fenton chemistry in which Fe(II) and H_2O_2 react to form hydroxyl radicals, highly reactive oxidants that are capable of depolymerizing cellulose and hemicellulose. The availability of the *P. placenta* genome also offers insight into the phylogeny of lignocellulose- degrading fungi. For example, comparisons between *P. placenta* and the closely related white rot fungus, *Phanerochaete chrysosporium*, support an evolutionary shift from white rot to brown rot, during which genes and gene families were lost, including those encoding lignin peroxidases and manganese peroxidases which are required for depolymerization of lignin.

101. Mesosynteny between fungal chromosomes; a newly recognised type of sequence conservation found between fungal species.
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As new fungal genomes are sequenced and assembled, it becomes feasible to compare features between species. We have compared the Dothideomycetes, seeking patterns of genome conservation. We compared the nucleotide and predicted peptide sequence content of all scaffolds of the Dothideomycete wheat pathogen *Stagonospora nodorum* and other available assemblies. To our surprise, we observed a highly non-random pattern of sequence conservation. There was a strong tendency for coding regions in one scaffold to be grouped in one or a few scaffolds of other species. The order and orientation of the genes was heavily scrambled. This pattern, which was striking within the Pleosporales, was also noticeable in other Pezizomycetes but not yeasts. We call this pattern mesosynteny to distinguish it from macrosynteny such as observed between cereal chromosomes and microsynteny, which refers to the conserved gene-by-gene order and orientation. We observed mesosynteny with both heterothallic and homothallic species, but much less so with asexual species. The conservation of mesosynteny implies an evolutionary advantage to retaining sets of genes on one chromosome over evolutionary time. It allows the gene content of ancestral chromosomes to be determined. It can be used as a genome level phylogenetic tool. It also has implications as a potential short-cut in genome sequencing and assembly.

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102. Outstanding: the dispensable chromosomes of *Mycosphaerella graminicola*

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Analysis of two genetic linkage maps of the wheat pathogen *Mycosphaerella graminicola* identified dispensable chromosomes that were present in both parents but absent in 15-20 % of the progeny. These Copy number Polymorphisms (CNPs) were confirmed with a Comparative Genomic Hybridization whole-genome array based on the finished genome of *M. graminicola* (http://genome.jgi-psf.org). Chromosomes 14- 21 were frequently absent among isolates, without visible effect on viability or virulence, whereas chromosomes 1-13 were invariably present. Genetic analyses showed that CNPs arises during meiosis, usually from nondisjunction at anaphase II. Overall, *M. graminicola* has the highest number of dispensable chromosomes reported. Varying from 0.41 to 0.77 Mbp, they comprise 38% of the chromosome number and 11.6% of the genome. The dispensable chromosomes are smaller and have significantly lower gene densities. Most of their genes are duplicated on the essential chromosomes and show a different codon usage. Dispensable chromosomes also contained a higher density of transposons, pseudogenes, and unclassified genes, which could encode novel proteins. Moreover, the dispensable chromosomes show extremely low synteny with other Dothideomycete genomes. We hypothesize that the dispensable chromosomes of *M. graminicola* are adaptive in some yet unknown way.

103. Detection of genes under positive selection in a complex of fungal species specialized on different host plants

G. Aguileta, T. Giraud

The most widely used indicator of positive selection is an excess of nonsynonymous codon substitutions between species that alter amino-acid sequence relative to the number of synonymous fixed differences and scaled to the number of such available sites. By this criterion, numerous genes in very diverse organisms have been shown to be under a positive selection pressure, especially when these genes are involved in reproduction, adaptation to contrasting environments, host-parasite interactions, and hybrid inviability. Looking for genes under positive selection in pathogens has triggered interest for some time, to elucidate the evolutionary dynamics of coevolution, and to develop vaccines or drugs that do not target rapidly evolving regions. No study so far has looked for genes evolved under positive selection among closely related pathogens specialized on different hosts, to elucidate the nature of genes involved in host specialization. We sequenced 40,000 ESTs from each of four Microbotryum species (responsible for anther smut disease on Caryophyllaceae), specialized on different host plants. Ca. 10% of the genes were found to evolve under positive selection, and many were likely to be involved in nutriment uptake from the host plants, secondary metabolite synthesis and secretion, respiration under stressful conditions and stress response, hyphal growth and differentiation, and regulation of other genes. Many of these genes have transmembrane domain and may therefore also be involved in addition in the recognition of the pathogen by the host. These functions are indeed likely to be involved in coevolution with the host and especially specialization onto new hosts.

104. The eisosome is a fungal innovation associated with the spatial regulation of endocytosis

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Endocytosis is a key cellular process in all eukaryotic cells. Its evolutionary innovation was therefore a pivotal moment in the origin of the eukaryotic cell. Eisosome organelles of budding yeast *Saccharomyces cerevisiae* localise to specialised domains on the plasma membrane, where they are thought to function in membrane remodelling, and the spatial regulation of endocytosis. In contrast, comparative genomics, coupled with cell biological studies reveals that the eisosome organelle is a fungal-specific innovation, with divergent forms present across the fungal kingdom. These large, immobile, assemblies are composed of several thousand copies of two paralogous proteins, Pil1 and Lsp1. Here, we describe the functional characterization of putative eisosome components in the filamentous ascomycete fungus, *Magnaporthe oryzae*. We have used targeted gene-deletion to genetically dissect the role of eisosome-associated proteins in this important plant pathogenic fungus, and fluorescent protein fusions to demonstrate the differential localisation of these proteins during infection-related development. In spores of the rice blast fungus, a Pil1-GFP fusion protein localises to punctate patches at the cell periphery, in a pattern consistent with that of eisosomes. Interestingly, the spatial distribution of Pil1-GFP is radically different in vegetative hyphae and invasive hyphae, which are used by the fungus to proliferate within living plant tissue. This suggests that endocytic mechanisms may be distinct in these two developmental stages. Targeted gene-deletion of *MoPIL1* results in aberrant membrane invaginations and associated defects in cell wall biogenesis.

105. Evidence for Hybridization and Introgression between *Coccidioides immitis* and *C. posadasii*

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Coccidioides is a soil-dwelling dimorphic fungus endemic to semi-arid areas of the Americas. This pathogen of humans and other mammals was chosen for comparative genomics at the species and population levels. Sequenced strains represent both species, with variation in biogeography, virulence, and isolate source. The nature of the species and population boundaries are of particular interest because of our recently discovered evidence of hybridization and introgression between species. Evidence for gene flow across the two species was detected by reciprocal blastn in all pairwise combinations for nonredundant 1kb fragments from four *C. immitis* and three *C. posadasii* genomes. Our analysis identified regions from each genome that had a closer match to the opposite species than its own. For most of the *C. posadasii* strains, there were few regions that had a closer match to *C. immitis*. However, there was one region within 3 of the 4 *C. immitis* strains that matched *C. posadasii*. This region contains a common border and a variable border. To assess the frequency of this *C. posadasii*-type region in *C. immitis*, a larger collection of isolates was screened. Approximately 30% of the *C. immitis* isolates contain this 70-120kb region, and the majority of those are from the southern California and Mexico populations. Our results suggest that hybridization has occurred between species, and at least one fragment appears to have introgressed in *C. immitis.* Additional Solexa sequencing confirms the observation.

106. Comparative Genome Browser: Initiative Bioinformatics Platform for Fungal Comparative Genomics.

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Since the whole genome sequences of yeast were released in 1996, 210 fungal genomes from 101 fungal species including 6 oomycetes have become publicly available. Around 100 genomes of multiple strains of fungi as well as heterogeneous formats of genome sequences address needs of standardized tools for fungal comparative genomics. To satisfy the needs, comparative genome browser (CGB) was developed on the basis of the Seoul National University Genome Browser (http://genomebrowser.snu.ac.kr/). The similar regions on the genomes of the same species were calculated and stored in CGB. CGB provides an interactive web interface for the display of the genome-wide alignments, allowing the user to assess multiple genomes at once via the same user interface with seven different display formats. It also supports the text browser function for displaying nucleotide sequences for the selected region, permitting users to confirm single nucleotide polymorphisms among the selected genomes. CGB will be the initiative platform for comparative genomics in fungal kingdom.

107. Evolution of sex in fungi

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Sexual reproduction is nearly universal and yet occurs in myriad forms throughout nature. We are addressing sex in the fungal kingdom (1) focusing on three themes. First, the structure, function, and evolution of the mating type locus (MAT) that governs cell identity during sexual reproduction, and serves as a model for the evolution of gene clusters and sex chromosomes. Second, transitions in sexual reproduction between out-crossing tetrapolar and inbreeding bipolar mating systems, and heterothallic and homothallic mating, including the emergence of unisexual reproduction in some species. Third, exploration of the evolutionary trajectory of Candida pathogenic species in which some have retained complete sexual cycles, including meiosis, whereas others have adopted parasexual cycles in which meiosis is absent or cryptic.

(1) Sex in Fungi: Molecular Determination and Evolutionary Implications, ASM press, 2007.

108. Progress under the Fungal Genome Initiative : Sequencing and comparative analysis of fungal genomes

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The Fungal Genome Initiative was launched in 2002 to generate genomic resources including fungal genome sequences, annotations, and analytic tools to support research on organisms across the fungal kingdom. To date the FGI has released genome sequences for 57 fungi, including fungi from all four major branches of the fungal tree. In addition, 9 approved fungi are currently in the sequencing queue. A primary focus of the FGI has been to promote comparative studies by sequencing clusters of related fungi and phenotypic variants of species to help interpret the biology of key models or pathogens. Recently, major sequencing efforts have targeted clusters of species as well as multiple strains of human pathogenic fungi. These species, including Candida, dermatophytes, and the dimorphic pathogens (*Coccidioides*, *Paracoccidioides*, *Blastomyces* and *Histoplasma*) have been selected for comparative analysis to identify genomic attributes that could contribute to the pathogenesis of these highly infectious fungi. We will describe recent progress on analysis of these genomes as well as new tools and web sites to help make these comparative data most useful to the community. We will also address how we are applying new sequencing technologies to produce new genome assemblies and look at genome-wide variation.

109. Dissecting pathogenicity of *Phytophthora ramorum* through comparative genomics.

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Sudden Oak Death, caused by *Phytophthora ramorum*, kills oak and other tree species. This threatens the integrity and biodiversity of forests in the USA. Due to its significance, a genome sequence of the pathogen was published in 2006. *Phytophthora* is a member of a class Oomycetes, which belongs to a distinct kingdom Stramenopila. The Stramenopila lineage diverged from major kingdoms of eukaryote, i.e., Animals, Fungi, and Plants in the remote past. Because of this reason, a large part of *Phytophthora* genome is distinct from genomes of model organisms such as *Arabidopsis*, *Drosophila* and *Saccharomyces*. Nevertheless, Oomycetes are composed of mycelia and they feed on decaying matter, just like fungi. It is not clear if this resemblance of fungi and Oomycetes is a consequence of a convergent evolution or alternatively, resulted from homologous genetic mechanisms. Taking the advantage of abundant Eukaryotic genome data, we systematically conducted comparative genomics and examined phylogenetic distribution of gene homologs. We are specially interested in genes which are conserved between Oomycetes and fungi but are diverged or undetectable in the genomes of animals, plants or diatoms (Stramenopila). It was found that 116 genes are shared exclusively between Oomycetes and fungi. In addition, functional category analysis revealed that genes for "polysaccharide metabolism" were overrepresented in this subset. We will discuss gene regulation and possible functions of the Fungal/Oomycetes specific genes.

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110. Histidine kinase two component response regulator proteins regulate reproductive development, virulence, and oxidative/osmotic stress responses of *Cochliobolus heterostrophus* and *Gibberella zeae*

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Two-component histidine kinase (HK) phosphorelay signaling systems are a major mechanism by which organisms sense and adapt to their environment. In response to a signal, the HK autophosphorylates a conserved histidine, then the phosphate is transferred to a conserved aspartic acid residue in a response regulator (RR) protein, resulting in an output. Nearly all eukaryotic HKs are hybrid with the HK and the RR domains in a single polypeptide; most characterized hybrids require an additional phosphorelay step through a histidine phosphotransfer (HPT) domain protein and a second RR. This additional phosphorelay step may allow the organism to integrate multiple input signals into a single output. The maize pathogen, *Cochliobolus heterostrophus* has 21 HKs, 4 RRs (*SSK1, SKN7, RIM15, REC1*) and one *HPT* gene. Because all HKs signal through RRs, we made RR gene deletion mutants and screened for altered phenotypes (virulence, asexual and sexual development, stress responses, drug resistance). We report that *C. heterostrophus* and *G. zeae* Ssk1p are required for virulence to maize and wheat, respectively. Lack of Ssk1p affects female fertility of heterothallic *C. heterostrophus*, self-fertility and ascospore maturation of homothallic *G. zeae*, and proper timing of sexual development in both. Crosses involving *C. heterostrophus ssk1* mutants ooze masses of ascospores prematurely, and tetrads cannot be found. Ssk1p also controls conidium proliferation and regulates the ratio of asexual to sexual spore development in the sexual reproductive phase in both fungi. Double *C. heterostrophus ssk1skn7*-deletion mutants are more sensitive to oxidative/ osmotic stress and more exaggerated in their spore-type balance phenotype than single *ssk1* and *skn7*-deletion mutants. *ssk1*-deletion mutant phenotypes largely overlap with *hog1*-deletion mutant phenotypes, allowing us to place *SSK1* upstream of the *HOG1* MAPK pathway.

111. Development of genomic resources and tools for *Cochliobolus sativus*

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Cochliobolus sativus is the causal agent of three economically important diseases of barley and wheat: spot blotch, common root rot, and black point. To clone and characterize virulence genes in *C. sativus* and develop an understanding of the genetic and molecular interactions with the two major hosts (barley and wheat), we developed various genomic resources and tools for the fungus. Fungal crosses were made between two isolates (ND90Pr and ND93-1) of *C. sativus* that exhibit a differential virulence pattern on barley and wheat and classical genetic analysis showed that the virulence on barley and wheat is controlled by a single gene locus, respectively. A molecular map of *C. sativus* was constructed using AFLP and RFLP markers and six DNA markers were identified to be associated with a virulence locus for barley. Pulse field gel electrophoresis combined with telomere analysis indicated that *C. sativus* has a genome size of 33 Mb. A Fosmid DNA library was constructed for each of the two parental isolates (ND90Pr and ND93-1) with a 16X genome coverage. A bacterial artificial chromosome (BAC) library was also constructed for the isolate ND90Pr and 7200 clones with an average insert size of 110 kb are available for gene screening. In addition, more than 8,000 cDNA clones were sequenced and 7,200 ESTs were generated. Protoplast-mediated transformation system has been developed for gene knock out and for gene function characterization. RNA-mediated gene silencing system based on a Gateway cloning was established and optimized using the green fluorescence protein (GFP) gene and the Tox A gene from the wheat tan spot pathogen, *Pyrenophora tritici-repentis*. With the development of these genomic resources and tools, we are cloning and characterizing genes of interests in *C. sativus*.

112. The evolution of necrotrophic parasitism in the Sclerotiniaceae.

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The Sclerotiniaceae (Ascomycotina; Leotiomycetes) is a promising system for studying the functional and genomic aspects of the evolution of trophic type; members of the family demonstrate the range of obligate host specificity to host generalism. To examine the differences between necrotrophy and biotrophy, we are screening for the presence of 8 genes involved in host cell death in a panel of isolates representing the trophic spectrum. The comparison between pathogenicity-related gene trees and a phylogeny inferred from "neutral" loci, as well as *in planta* gene expression, will elucidate how the exemplar taxa differ in regulation and expression patterns of pathogenicity-related genes. We have inferred an expanded phylogeny of the Sclerotiniaceae generated from the combined sequence of heat shock protein 60, glyceraldehyde-3-phosphate dehydrogenase, and calmodulin. Based on this phylogeny, biotrophy appears to be the derived state, with at least two shifts from necrotrophy to biotrophy. Our candidate genes code for pathogenicity related proteins involved in: i.) cell wall degradation (acid protease 1, aspartyl protease, exopolygalacturonase 1, and endopolygalacturonses 3, 5, and 6); and ii.) the oxalic acid pathway (oxaloacetate acetylhydrolase, and zinc finger transcription factor, Pac1). Gene genealogies were inferred from both primary and amino acid sequences. These were compared to the expanded phylogeny to establish if the pathogenicity-related loci are under selection. The comparisons between the gene and protein phylogenies provided evidence for selective constraints for specific taxa, and rate- and site-specific likelihood models were used to identify positive or purifying selection in these genes. Preliminary expression data will be presented.

Gene Regulation

113. Post translational modification of AoXlnR, a key transcriptional regulator of biomass- degrading enzymes in *Aspergillus oryzae*.
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We previously identified *A. oryzae* XlnR (AoXlnR) as a transcriptional regulator of cellulase and xylanase genes. Anlalysis using *A. oryzae* oligonucleotide array revealed that it activated transcription of 38 enzyme genes possibly involved in cellulose and xylan degradation and D-xylose metabolism upon induction by D-xylose. Since cellulose and xylan are two major plant polysaccharides, AoXlnR appears to be a most important regulator of biomass degrading enzyme genes. The purpose of our current research is to clarify the molecular mechanism of AoXlnR- mediated transcriptional regulation. We found that AoXlnR was reversibly modified in the presence of D-xylose. Calf Intestine Alkaline Phosphatase treatment revealed that it was phosphorylated both in the presence and absence of D-xylose, and that the degree of phosphorylation was higher in its presence. Furthermore, AoXlnR in the presence of D- xylose had a higher molecular weight than that in its absence even after the phosphatase treatment. These results indicated that AoXlnR is constitutively phosphorylated and that D-xylose triggers additional phosphorylation and also an unknown modification. We are now trying to identify the phospholyration sites and the unknown modifier.

114. Substitutions in amino terminal tail of histone H3 cause dominant loss of DNA methylation in Neurospora
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In Neurospora DNA methylation depends on methylation of histone H3 lysine 9 (H3K9me) by the SET domain methyltransferase, DIM-5. Heterochromatin protein HP1 reads the trimethyl mark and directly recruits the DNA methyltransferase DIM-2 to regions destined for DNA methylation. Here we elucidate the role various amino acid residues of H3 on methylation of H3K9 and DNA. A single gene codes for histone H3 in Neurospora (*hH3*). We noted earlier that introduction of an ectopic H3 gene bearing a substitution at K9 caused dominant global loss of DNA methylation and reactivation of a silenced selectable marker (*hph*). We observed similar loss of DNA methylation for other alleles including those encoding R2L, A7M, R8A, S10A, T11A, G12P, G13M, K14Q and K14R. Except for R2L, K14Q and K14R these substitutions also resulted in a loss of DIM-5 activity *in-vitro*. In addition, we show that DIM-5 is sensitive to methylation of H3K4 and phosphorylation of H3S10. To test recessive effects of histone mutations, we used a null allele of histone H3 generated using RIP ($hH3^{RIP1}$) to create Neurospora strains that only have the desired substitution. The inability to isolate viable strains with only mutant version for some suggests that the corresponding residues are essential. In summary, we will present results suggesting that the amino terminal tail of histone H3 acts as a platform to integrate various signals to influence the methylation of H3K9 by DIM-5 and the ability of HP1 to read this trimethyl mark in order to regulate DNA methylation.

115. The action of Neurospora region-specific recombination regulators is regulated by MSUD.
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rec 1+, rec 2+ and *rec 3+* are dominant trans-acting genes that suppress meiotic recombination in specific regions of the *Neurospora crassa* genome. For example, up to 1% of progeny from a *rec 2* by *rec 2* cross experience recombination in *his 3* but this falls to ~0.005% when one or both parents carries *rec 2+*. RFLP mapping after a long walk along chromosome V indicated that *rec 2+* is in a 10kb stretch of DNA that is absent from *rec 2* strains which have in its place a 3kb stretch of unique DNA. This has been confirmed by sequencing a *rec-2* strain with the Solexa genome analyser. Oddly however, putting *rec 2+* DNA into *rec 2* strains failed to yield a *rec 2+* phenotype while deletion of *rec 2+* DNA failed to yield a *rec 2* phenotype. We now know that MSUD is responsible for the dominance of all three *rec+* genes, resolving this puzzle. For example, disabling MSUD in *rec 2+* by *rec 2* heterozygotes increases recombination at *his 3* and deletion of *rec 2* leads to low recombination. Thus, rather than rec+ genes producing suppressors of recombination, the products of *rec 1*, *rec 2* and *rec 3* act to promote recombination in specific regions of the genome. We are currently using the Solexa system to identify *rec-1* and *rec-3*.

116. DCL-1 colocalizes with other components of the MSUD machinery and is required for silencing
William G. Alexander, Namboori B. Raju, Hua Xiao, Thomas M. Hammond, Tony D. Perdue, Robert L. Metzenberg, Patricia J. Pukkila, and Patrick K.T. Shiu

In Neurospora, a gene present in an abnormal number of copies is usually a red flag for mischief. One way to deal with these potential intruders is by destroying their transcripts. Widely known as RNA interference (RNAi), this mechanism depends on the ''dicing'' of a double-stranded RNA intermediate into small-interfering RNA, which in turn guide the degradation of mRNA from the target gene. Here, we show that Meiotic Silencing by Unpaired DNA (MSUD), a mechanism that silences expression from unpaired DNA during meiosis, requires the dcl-1 (but not the dcl-2) gene for its function. This result suggests that MSUD operates in a similar manner to Quelling and other RNAi systems. We also show that DCL-1 colocalizes with SAD-1 (an RdRP), SAD-2, and SMS-2 (an Argonaute) in the perinuclear region.

117. Transcriptome analysis of the *Aspergillus fumigatus* calcineurin

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Calcineurin plays an important role in the control of cell morphology and virulence in fungi. Calcineurin is a serine/threonine-specific phosphatase heterodimer consisting of a catalytic subunit A and a regulatory Ca2+/Calmodulin binding subunit. A mutant of *A. fumigatus* lacking the calcineurin A (*calA*) catalytic subunit exhibited defective hyphal morphology related to apical extension and branching growth, which resulted in drastically decreased filamentation. Here, we investigated which pathways are influenced by *A. fumigatus* calcineurin during proliferation by comparatively determining the transcriptional profile of *A. fumigatus* wild type and delta *calA* mutant strains. Our results showed that although the mitochondrial function is reduced in the delta *calA* mutant strain, its respiratory chain is functional and the mutant has increased alternative oxidase (*aoxA*) mRNA accumulation and activity. Furthermore, we identified several genes that encode transcription factors that have increased mRNA expression in the delta *calA* mutant and that could be involved in the Cal-CrzA pathway. Deletion mutants for these transcription factors had also reduced susceptibility to itraconazole, caspofungin, and SDS. *A. nidulans* calcineurin overexpression induced an augmentation of the germ tube lenght.

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118. Activation of silent gene clusters in *Aspergillus nidulans*

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Microorganisms as bacteria and fungi produce important low-molecular weight molecules that show different biological activities. Genome mining of available fungal genomes indicated that their potential to produce these compounds designated secondary metabolites (SMs) is greatly underestimated. Fungi encode the genetic information for the biosynthesis of many more compounds which still await discovery. The vast amount of DNA sequence in the public database represents only the beginning of this new genomics era. Most of the fungal secondary metabolism gene clusters are silent under laboratory conditions. Despite this limitation, to get access to the vast number of unknown compounds encoded by silent gene clusters, mixing genomic data, genetic engineering and analytical techniques provides a new avenue to discover novel and potentially bioactive natural products. Bioinformatic analysis of the published *A. nidulans* genome sequence led to the identification of 48 putative SM gene clusters. By overexpressing a pathway-specific transcription factor, we were able to induce a silent gene cluster containing the gene for a mixed PKS/NRPS system. This approach is rendered feasible by the fact that all of the genes encoding the large number of enzymes required for the synthesis of a typical secondary metabolite are clustered and that in some cases, a single regulator controls the expression of all members of a gene cluster to a certain extent. By this way, we were able to isolate novel compounds. In addition, we will discuss further attempts to activate silent gene clusters and to identify physiological conditions under which gene clusters are active under natural conditions.

119. Chromatin-remodeling and epigenetics at the *frequency* locus

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Circadian-regulated gene expression is controlled by a transcriptional negative feedback loop and a recent body of evidence indicates that epigenetics and chromatin- remodeling are integral to this process. We have previously determined that multiple ATP-dependent chromatin-remodeling enzymes function at the clock gene *frequency (frq)*. We now report that the Neurospora homologue of a chromodomain helicase DNA-binding (*chd2*) gene is required for epigenetic transfer of time and remodels chromatin at the *frq* locus. Specifically, there seems to be a major defect in remodeling at the *frq* antisense promoter in the *Delta-chd2* strain. Unexpectedly, our studies on CHD2 activity revealed that DNA sequences within the *frq* promoter are typically methylated and are hypermethylated in *Delta-chd2* strains. Normal WT DNA methylation requires both a functional circadian clock as well as the *frq* antisense transcript, *qrf*, indicating that clock components contribute to the regulation of DNA methylation. Furthermore, the DNA methyltransferase, DIM-2, is required for *frq* methylation. Phenotypic characterization of *Delta-dim-2* strains shows that DNA methylation is not necessary for rhythms, although these strains display a minor phase advance of approximately 2 hours suggesting that promoter methylation may be used to fine tune gene expression.

120. Laccase and polyketide synthase affect virulence and sporulation in the corn smut pathogen *Ustilago maydis*

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Ustilago maydis, a maize pathogen, must produce teliospores to complete its life cycle. Teliosporogenesis normally occurs only *in planta*. However, deletion of *ust1*, encoding an APSES domain protein, yielded a striking phenotype including the production of highly pigmented spore-like structures in culture. Therefore, the *ust1* mutant provides a host free genetic system to begin to decipher the sporulation associated transcriptome. To this end we are utilizing microarray analysis of the *ust1* mutant for comparison with in planta sporulation. Analysis showed 36 genes upregulated 20 fold or higher in the *ust1* mutant. One of these genes is *ssp1*, previously published as specifically and highly expressed in teliospores. This confirms that the *ust1* is a good surrogate for sporulation. We have thus far focused on two of these upregulated genes, laccase and polyketide synthase. Laccase because of its role as a cell wall-associated virulence factor and melanin biosynthesis of the spore wall in *Cryptococcus neoformans*. Polyketide synthase due its requirement for virulence and T-toxin production in *Cochliobolus heterostrophus* and its involvement in melanin biosynthesis pathway of the phytopathogenic fungus *Bipolaris oryzae*. We generated a laccase deletion mutant, which exhibit delayed pathogenicity. We generated a polyketide synthase deletion mutant and our preliminary results showed it to be dramatically impaired in pathogenicity. Interestingly, galls of the polyketide synthase mutant also showed reduced teliospore production. This project is supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2006-35319-16738.

121. Cell wall integrity MAP kinase signalling pathway in the human-pathogenic fungus *Aspergillus fumigatus*

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Mitogen-activated protein kinase (MAPK) cascades are evolutionary conserved signalling modules transducing stimuli from the cell surface to the nucleus. MAPK pathways control key virulence functions and are involved in the regulation of various cellular responses in eukaryotes. In *A. fumigatus*, three putative MAP kinases are involved in the cell wall integrity (CWI) signalling pathway: *bck1* (MAPK kinase kinase), *mkk2* (MAPK kinase) and *mpkA* (MAPK).To investigate whether *bck1*, *mkk2* and *mpkA* are involved in CWI signalling of *A. fumigatus*, mutant strains of respective genes were constructed and phenotypically analysed. In addition, transcriptional regulation of *mpkA*, and the function of the corresponding protein was studied. An *A. fumigatus* strain was generated carrying the *lacZ*- reporter gene fused to the *mpkA*-promoter. Post transcriptional modification of MpkA was monitored by western blot analysis.Functional analysis of Bck1, Mkk2 and MpkA in *A. fumigatus* revealed their involvement in hyphal growth and filamentation. The sensitivity of these mutants against cell wall inhibitors demonstrates that the genes are acting in the CWI signalling pathway and mediate cell wall damage responses. Furthermore, new insights into the regulation of MAPK signalling in *A. fumigatus* were gained. It was observed that *mpkA* is regulated transcriptionally in response to cell wall disrupting agents. By contrast, regulation of MpkA following treatment with oxidative stress agents occurs at the post transcriptional level. Interestingly, delta *mpkA* mutants are still virulent in a mouse infection model.

122. A complex photoreceptor system mediates gene photoactivation in *Neurospora crassa*.

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Genes *con-10* and *con-6* in *Neurospora crassa* are activated during conidiation or after illumination of vegetative mycelia with blue light.We have determined the threshold for gene activation by light, and the kinetics of mRNA accumulation and degradation after a light stimulus.The threshold for *con-10* and *con-6* photoactivation is 10^2 J/m^2, much higher than that for other photoresponses in Neurospora.We have observed a complex stimulus/response relationship for *con-10* and *con-6* photoactivation that has led us to propose the existence of two photoreceptor systems optimized to operate at different light intensities. We investigated gene photoactivation in strains with deletions in photoreceptor genes or in genes that could be involved in the regulation of light responses (*wc-1*, *wc-2*, *cry-1*, *nop-1*, *phy-1, phy-2, ve-1*).The absence of gene photoactivation with low or high-intensity light in wc mutants confirmed that the White Collar Complex participates in the low and high intensity photoreceptor system.A deletion of the the blue-light photoreceptor cryptochrome resulted in high gene photoactivation and altered the shape of the stimulus/response curve without modifying the sensitivity to light. Light-dependent carotenoid accumulation was not altered in any of the mutants, with the expected exception of the *wc* strains, but the *ve-1* and the *phy-2* mutants accumulated less carotenoids than the wild type. Our results suggest that the cryptochrome modifies the regulatory activity of the White Collar Complex.

123. The *Aspergillus nidulans* phytochrome FphA binds to the promoters of two putative conidiation genes (*ccgA* and *conJ*).

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Light serves as an important environmental signal to regulate development and metabolism in many fungi and has been studied to some detail in *Neurospora crassa* and *Aspergillus nidulans*. *A. nidulans* develops mainly asexually in the light and mainly sexually in the dark. The red-light sensor phytochrome (FphA), and the WC-1 homologue blue-light receptor LreA have been shown to be functional in *A. nidulans* and mediate the light response. There is evidence that both proteins form a light regulator complex (LRC) to control the expression of different genes*. LreB (WC-2) and VeA are probably also components of this protein complex*. Using Chromatin immunoprecipitation (ChIP) we show that HA-tagged FphA binds to the promoters of *N. crassa* homologues *conJ* (*con-10*) and *ccgA* (*ccg-1*). Experiments to determine the functions of these two genes in *A. nidulans* are under way. We found that FphA binds preferentially in the light to these two promoters. We also tested whether FphA binds to the promoters of other genes with well-known functions in asexual development of *A. nidulans*, such as *brlA*, *fluG*, and *flbA*. Precipitation of FphA did not precipitate any of those promoters, suggesting that these genes are not direct targets of the light regulator complex. *J. Purschwitz, S. Müller, R. Fischer, *Mol Genet Genomics* (2008).

124. Farnesol induces the transcriptional accumulation of the *Aspergillus nidulans* Apoptosis- Inducing Factor (AIF)-like mitochondrial oxidoreductase.

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Farnesol (FOH) is a non-sterol isoprenoid produced by dephosphorylation of farnesyl pyrophosphate, a catabolite of the cholesterol biosynthetic pathway. These isoprenoids inhibit proliferation and induce apoptosis. It has been shown previously that FOH triggers morphological features characteristic of apoptosis in the filamentous fungus *Aspergillus nidulans*. Here, we investigate which pathways are influenced through FOH by examining the transcriptional profile of *A. nidulans* exposed to this isoprenoid. We observed decreased mRNA abundance of several genes involved in RNA processing and modification, transcription, translation, ribosomal structure and biogenesis, amino acid transport and metabolism, and ergosterol biosynthesis. We also observed increased mRNA expression of genes encoding a number of mitochondrial proteins and characterized in detail one of them, the aifA, encoding the Apoptosis- Inducing Factor (AIF)-like mitochondrial oxidoreductase. The delta *aifA* mutant is more sensitive to FOH (about 8.0% and 0% survival when exposed to 10 and 100 microM FOH respectively) than the wild type (about 97% and 3% survival when exposed to 10 and 100 microM FOH respectively). These results suggest that AifA is possibly important for decreasing the effects of FOH and reactive oxygen species.

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125. Expression of genes encoded at chromosome ends during sporulation in the corn smut pathogen *Ustilago maydis*
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To identify genes implicated in sporulation in *U. maydis* we are utilizing microarray analysis of the *ust1* mutant. This mutant produces highly pigmented spore-like structures in culture. We observed a gene family of 23 related genes that appeared highly induced in the *ust1* mutant. Interestingly, all 23 genes were located at chromosome ends. These genes were noted in publications on gene families encoded at chromosome ends (Kamper et al., 2006; Sanchez-Alonso et al., 2008). All genes in this gene family have overlapping homology. The homology amongst them ranges from 78% to 98%. The high conservation of the probe sets designed for these 23 *U. maydis* genes confounds our microarray analysis due to cross hybridization. To determine if all or only a subset of these genes is induced upon sporulation, real time PCR with gene specific primer sets is underway to differentiate individual member transcription. This project is supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2006-35319-16738.

126. Characterization of small RNAs from mycelia and appressoria of *Magnaporthe oryzae*
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Magnaporthe oryzae causes blast disease on rice and is a leading model fungus to study plant-pathogen interactions. Recently, new insights into the mechanisms by which *M. oryzae* causes disease has been elucidated through genomics and transcriptome studies. Small non-coding RNAs (ncRNAs) such as small interfering RNAs (siRNAs), repeated-associated siRNAs (rasiRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs) play important functional roles in plants and animals. However, their presence and role in fungi is largely unknown. In this study, we generated deep sequence coverage of sRNAs from mycelia and appressoria that resulted in a total of 95,570 non-redundant sRNAs greater than 16 nucleotides long. After normalization of the data, ~ 45.0% of sRNAs mapped to rRNA in both libraries. Furthermore, about 14.0% of sRNAs from mycelia mapped to repeats such as MAGGY in contrast to only 2.3% in appressoria. Notably, sRNAs mapped to both sense and antisense strands in approximately equal frequency. We also observed that the size of sRNAs for MAGGY had a peak length of 22 – 23 nucleotides; characteristics of siRNA. Our results suggest that expression of repetitive elements such as transposons were silenced in mycelia. In addition, we found ~ 19.5% of appressoria sRNAs mapped to tRNA in contrast to ~ 9.4% from mycelia. Our data, demonstrates that tRNA cleavage occurs at the tRNA anticodon and is tissue dependent suggesting that tRNA processing may regulate protein synthesis. In conclusion, evidence suggests different classes of sRNAs exist in *M. oryzae* and their frequency profiles in different tissues suggest they may play an active role in growth and development. However, this requires further investigation.

127. Fumonisin-nonproducing mutants exhibit differential expression of putative polyketide biosynthetic gene clusters in *Fusarium verticillioides*.
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The maize pathogen *Fusarium verticillioides* produces a group of polyketide derived secondary metabolites called fumonisins. Fumonisins can cause diseases in animals and have been correlated epidemiologically with esophageal cancer and birth defects in humans. The fumonisin biosynthetic gene cluster (*FUM*) is transcriptionally regulated by a zinc binuclear DNA binding protein encoded by *FUM21*. Deletion of *FUM21* results in the absence of *FUM* gene expression and fumonisin production. Additionally, deletion of another transcription factor, *ZFR1*, leads to a reduction in fumonisin production. Lack of fumonisin production leads to altered expression of a number of putative gene clusters containing polyketide synthase genes. A survey of gene expression, BLAST homology prediction and genomic location allowed for a description of each putative gene cluster. Besides the fumonisin polyketide synthase gene, the functions of only three other *F. verticillioides* polyketide synthases have been characterized and concomitant biosynthetic product identified. Differential expression of putative gene clusters involved in the production of bikaverin, fusarin and a perithecial pigment are described. The identification of growth conditions coupled with a fumonisin deficient strain might allow the rapid identification of novel metabolites produced by the uncharacterized, putative secondary metabolite gene clusters and help to elucidate the biological role of their metabolites.

128. The Relationship between *Tri10* and *Tri6* in *Fusarium graminearum*
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The *Fusarium* species is responsible for a $2.7 billion loss in the North American agriculture industry in the 1990s. Fungal pathogens belonging to this genus are renowned for causing blight and brown rot on cereal crops such as wheat and barley, thus reducing crop yield, as well as contaminating crops with mycotoxins hence lowering crop quality. Production of such mycotoxins, also known as trichothecenes, is controlled by the trichothecene biosynthesis pathway, with two known positive regulators *Tri6* and *Tri10*. In *Fusarium sporotrichioides*, *Tri10* had been shown to act upstream of *Tri6*. However, the relationship between *Tri10* and *Tri6* has not been established in *Fusarium graminearum (Fg)*. In this poster, the relationship between Tri10 and *Tri6* in *Fusarium graminearum* will be described through targeted gene disruption studies and yeast two-hybrid analysis.

129. Discovery of multiple signal transduction pathways involved in generating circadian rhythms
Lindsay Bennett and Deborah Bell-Pedersen. TAMU

In Neurospora crassa, the circadian clock impinges upon the Osmolarity Sensing pathway (OS), an evolutionarily conserved mitogen activated protein kinase (MAP kinase) pathway to regulate clock controlled genes. The clock signal is transduced through the rhythmic phosphorylation of the MAP kinase OS-2. Neurospora contains two additional MAP kinase pathways and here we show that the MAP kinases, MAK-1(MKC-1) and MAK-2, are also rhythmically phosphorylated. We are currently investigating whether these rhythms in phosphorylation are dependent upon the FRQ/WCC oscillator and identifying target genes of these pathways. MAK-1 is a homologue of Slt2, the MAP kinase responsible for cell wall integrity in yeast. We are testing whether several clock controlled genes predicted to be involved in cell wall synthesis or integrity, which are also known to be regulated by Slt2 in yeast, are also regulated by MAK-1. Furthermore, MAK-2, a component of the signaling cascade which facilitates mating, is a repressor of ccg-4, which encodes a pheromone precursor. Thus, the clock may be acting through MAK-2 to generate rhythms in ccg-4. We hypothesize that by co-opting established signaling pathways, circadian oscillators are better able to efficiently regulate multiple genes that prepare the organism for specific environmental changes.

130. Regulation by light of conidiation genes in *Aspergillus nidulans*

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The ascomycete fungus Aspergillus nidulans is a model organism for the research on conidiation. Red and blue light stimulate conidiation in A. nidulans, but mutations in the veA gene allow conidiation in the dark. Conidiation in A. nidulans is controlled by a master regulator, the product of the brlA gene. Many gene products act upstream of brlA, presumably allowing the synthesis of chemicals or allowing the transduction of environmental signals, to trigger brlA transcription and conidiation. The regulation of conidation by light may occur through activation of the expression of some regulatory genes. We have shown that the expression of several conidiation genes, including brlA, fluG flbA, flbB and flbC, is activated by light. The level of photoinduction was moderate, between 4-10 fold, and changed with the age of the culture and the genetic background. Some genes, like brlA, are activated by light regardless of age, but flbA was only induced by light in 18 h-mycelia, while flbC was only induced in 25 h-mycelia. Mutation in veA did not prevent photoactivation of gene expression, and an increase in light-dependent mRNA accumulation was observed for flbA and flbC. The veA mutant did show an increase in the accumulation of some conidiation mRNAs in the dark. The activation by light of conidiation genes would provide increased amounts of regulatory proteins for conidiation clarifying the role of light on conidation.

131. Genome-wide analysis of light-inducible responses reveals hierarchical light signaling in *Neurospora*

Chen-Hui Chen, Carol S. Ringelberg, Robert H. Gross, Jay C. Dunlap and Jennifer J. Loros

Light signals mediated by WC-1 and WC-2 working as the WCC are essential for most light responses in *Neurospora crassa*, but the molecular mechanisms underlying the light sensing cascades and the roles of other putative photoreceptors and light- sensitive transcription factors remain poorly characterized. Unsupervised hierarchical clustering of 90 microarrays used to probe light-induced RNAs reveals that 314 genes, 5.6% of the detectable transcripts, are either early or late light responsive. The WCC plays a dominant role in regulating both types of light responses, and VVD in regulating late responses. Additionally, Submerged Protoperithecia-1 (SUB-1), one of six GATA family transcription factors that include WC-1 and WC-2, is bound by the WCC, is early light- responsive and is essential for most late light responses. Bioinformatics analysis of promoters confirmed the known Light Response Element (LRE), as well as identifying a Late Light Response Element (LLRE) required for full-induction of late light responses. These data provide a synoptic picture of the transcriptional response to light, as well as illuminating the cis and trans-acting elements comprising the regulatory signaling cascade that governs this response.

132. Cullin 4 is required for DNA methylation in Neurospora

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Cullins form the backbone scaffold of multi-subunit E3 ubiquitin ligase complexes. In order to test the possible role of the Cullin 4 (CUL-4) complex in DNA methylation, we generated a null mutant of *cul-4* using RIP (*cul-4^{RIP1}*). This mutant displays defects in vegetative and sexual development and shows a complete loss of DNA methylation, absence of H3K9 methylation and mislocalization of HP1-GFP. These defects could all be complemented by the wild type gene or epitope tagged constructs. In order to identify the interacting partners, the complex of proteins associated with CUL-4 was purified and subjected to mass spectroscopy. This revealed the presence of other proteins, DIM-7, DIM-8 and DIM-9, which were previously identified in a genetic screen for mutants required for DNA methylation. We will present results of our study aimed towards understanding the role of CUL-4 and its associated proteins in regulation of H3K9 and DNA methylation in Neurospora.

133. The *fkhA* gene encoding a forkhead transcription factor is required for sexual development in *Aspergillus nidulans*.

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The sexual developmental process of *Aspergillus nidulans* is largely affected by the genetic and environmental factors. Here, we identified the *fkhA* gene which encodes a putative forkhead transcription factor homologous to the yeast *FKH1* gene that is involved in sexual development. Since little is known about the involvement of the forkhead protein in the developmental process in the filamentous fungi, including *A. nidulans*, we generated a deletion mutant of *fkhA* gene and analyzed. Indeed, no fruiting bodies or cleistothecia were found in the *fkhA* deletion mutant, suggesting that the *fkhA* gene is required for sexual development in *A. nidulans*. Furthermore, *A. nidulans* genome analysis revealed that there are six possible forkhead genes including the *fkhA* gene. To know whether the other forkhead genes could affect sexual or asexual differentiation process, the knock-out mutant construction of each gene is in progress. [This work is supported by the KRF grants 2007-313-C00668 and 2008-313-C00804.]

134. RRMA, an RNA binding protein with key roles in amino acid catabolism, nitrogen metabolism and oxidative stress

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RRMA is involved in posttranscriptional regulation of gene expression in *Aspergillus nidulans*. The *rrmA* mutations were selected based on changes of arginine/proline metabolism. *?rrmA* mutation results in slower growth and higher sensitivity to oxidative stress. Independently, the RRMA was found as the protein which specifically binds to the 3'UTR of *areA* transcript. AREA is the transcriptional factor involved in nitrogen metabolism and its transcript is highly unstable under conditions of nitrogen repression. To test the role of RRMA in the regulation of transcript stability, stability of (*areA*) mRNA and arginine catabolism genes transcripts (*agaA, otaA*) has been analysed. Analysis of the transcript stability under different growth conditions (nitrogen repression, nitrogen or carbon starvation, oxidative stress) has shown higher stability of *areA*, *agaA* and *otaA* transcripts in *?rrmA* strain, especially under nitrogen repression or oxidative stress conditions. This indicates that RRMA participates in regulation of mRNA degradation in response to distinct signals (nitrogen repression, oxidative stress). The phenotype of *?rrmA* mutation indicates that the protein might be involved in general mechanism of regulated mRNA decay.

135. An investigation into temperature compensation of the *Neurospora crassa* circadian clock.

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In the poikilothermic organism *Neurospora crassa* key circadian clock components include *frequency* (*frq*), its antisense counterpart *qrf*, as well as the PAS protein encoding genes *white collar-1* (*wc-1*), *wc-2* and *vivid* (*vvd*). A key characteristic of any circadian clock is that the speed with which the clock ticks is temperature compensated. This is especially important in organisms that cannot maintain their own core body temperature as it allows them to maintain a fairly constant period length in a wide range of environmental temperatures. Recently we discovered that the clock-controlled gene *vvd* is involved in maintaining temperature compensation of clock phase. Our data indicate that temperature dependent levels of *vvd* gene products are required for correct phasing of clock-controlled conidiation. In the absence of *vvd* the timing of conidiation is temperature dependent occurring later as the temperature decreases. Considering that the levels of *vvd* are important for temperature compensation of clock output, we have investigated the levels of other *Neurospora* clock components in an attempt to gain further insight into underlying mechanism of temperature compensation. Data will be presented on the levels along with the cellular localisation of *Neurospora* clock components at different temperatures.

136. MAT1-1-2 is essential for sexual development in the homothallic fungus *Gibberella zeae* and in the heterothallic fungus *Podospora anserina*

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In the self-sterile *P. anserina* sexual reproduction is controlled by a single mating- type locus carrying either one of 2 idiomorphs, *MAT1-1* or *MAT1-2*. The self–fertile *G. zeae* carries linked counterparts of the *P. anserina MAT* genes. While three of these encode transcription factors, the molecular function of the fourth gene *MAT1-1-2* is not elucidated yet. Since *MAT1-1-2* inactivation resulted in barren perithecia in *P. anserina* [1], we constructed a deletion mutant in the cereal pathogen *G. zeae*, which was similarly sterile. We are testing whether heterologous complementation of both mutants is possible. MAT1-1-2 is present in all Sordariomycetes analyzed to date. All MAT1-1-2 proteins contain a stretch of 17 aa with three invariant residues, P, P, F, which was proposed to define a new DNA-binding domain [2].To investigate MAT1-1-2 structure-function relationships we constructed 39 alleles by site-directed mutagenesis in *P. anserina*. Deletion of the PPF domain led to a mutant phenotype, confirming its role in protein activity. Only the 2 missense mutations, W193A and A347D, conferred a sterile phenotype.

1. Arnaise *et al.*, Genetics (2001) 159: 545 2. Debuchy *et al.*, Mol Gen Genet (1993) 241: 667

137. The Pc-ACE1 transcription factor from *Phanerochaete chrysosporium* contains a Cys and Ser-rich transactivation domain.

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We have previously isolated the transcription factor PC-ACE1. In *S. cerevisiae* its ortholog is involved in copper homeostasis while in *P. chrysosporium* Pc-ACE1 regulates the expression of target gene *mco1*. Here we looked for the transactivation domain in the coding sequence of Pc-ACE1. Fusion proteins generated by linking Pc-ACE1 sequences to the DNA binding domain of the yeast GAL4 transcription factor activate the transcription of a reporter gene linked in *cis* to the GAL4 DNA binding site. The full length Pc-ACE1 protein is a poor transactivator. Deletion of the N- terminal half of the Pc-ACE1 protein augments the protein's transcription function. The C- terminal portion of the protein between amino acid residues 322 to 490 is a potent transactivator. Interestingly, a Cys cluster is located in this region. Moreover, between amino acid residues 529 to 632, a milder transactivator site in a Ser rich region (30%) is present. We propose that the carboxy terminal region contains two transactivating sequences, one stronger that is Cys-rich, and the other, a milder one, which is Ser-rich. This work is supported by a FONDECYT grant N°1085236, and Vicerrectoría Adjunta de Investigación y Doctorado (VRAID).

138. Sex locus and virulence of zygomycetes and microsporidia, the basal fungi

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Sexual reproduction of fungi is orchestrated by a delimited chromosomal region known as the mating type or *sex* locus. This locus is also involved in virulence in some pathogenic fungi. In this study we defined the *sex* loci of two different basal fungal lineages, zygomycetes and microsporidia. A previous study showed that, in the zygomycete *Phycomyces blakesleeanus*, the *sex* locus encodes a high mobility group (HMG) transcription factor and is flanked by genes encoding a triose phosphate transporter (TPT) and an RNA helicase. We found that microsynteny of the *sex* locus [TPT, HMG, RNA helicase] is highly conserved across zygomycetous fungi including *Rhizopus oryzae* and *Mucor circinelloides* and excluding other fungal lineages (Basidiomycota, Ascomycota, and Chytridiomycota). Human pathogenic zygomycetes cause a life threatening infection, mucormycosis. We found that minus mating type and large spore producing isolates are more virulent in *Galleria mellonella*, providing a possible link between the *sex* locus and pathogenicity of mucormycosis. We also found that three microsporidians, *Encephalitozoon cuniculi*, *Enterocytozoon bieneusi*, and *Antonospora locustae*, harbor a similar syntenic *sex* locus. *R. oryzae* and *E. cuniculi* share a higher level of genomic architectural similarity based on relaxed synteny analysis compared to other fungal lineages, providing evidence these obligate intracellular pathogens are true fungi evolutionarily related to the Zygomycota. The presence of a *sex* related locus in microsporidian genomes suggests these obligate pathogens may have an extant sexual cycle and studies to address this and its possible relationship to pathogenesis are in progress.

139. Characterization of *Cryptococcus neoformans BUD16* homologue

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Light regulates physiology, development and behavior in many organisms including fungi. *Cryptococcus neoformans*, a heterothallic basidiomycetous yeast, can sense blue light via the Cwc1 and Cwc2 proteins and in turn negatively regulates the production of sexual filaments. To dissect this pathway, we conducted a suppressor screen utilized *Agrobacterium tumefaciens*-mediated transformation (ATMT) technique to identify mutants suppressing the mating phenotype of the *CWC1* overexpression strain which displayed no filaments under light illumination. Here, we report that the EG30 strain restored the filamentation to the wild-type level and the T-DNA was verified to insert at the promoter of a gene related to bud site selection that is homologous to the *Saccharomyces cerevisiae BUD16* gene. The *BUD16* gene was identified due to the random budding pattern in the homozygous diploid mutant background. The Bud16p protein is a predicted pyridoxal kinase which converts pyridoxal into pyridoxal 5[ǁ]-phosphate (PLP), the biologically active form of vitamin B6. In *C. neoformans*, we found that the *bud16* mutant produced mating filaments slightly more than the wild-type strain and the cell fusion efficiency was also increased, while the *BUD16* overexpression strain displayed fewer dikaryotic filaments. On the other hand, monokaryotic filamentation, another sexual process involved the same sex of *MAT*£\ cells, appeared unaffected in the *MAT*£\ *bud16* mutants; however, overexpression of the *BUD16* gene blocked this differentiation. Epistasis analyses further revealed that *BUD16* acted downstream of the *CWC1* gene. Based on our results, we conclude that *BUD16* function as a negative regulator downstream of the Cwc complex in the mating differentiation.

140. Ssn8, a *Saccharomyces cerevisiae* Mediator homologue, negatively regulates sexual development, invasive growth, and the production of melanin and capsule in *Cryptococcus neoformans*

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Cryptococcus neoformans is an important human pathogen and also a model for studying fungal physiology and differentiation. Our prior studies showed that blue light negatively regulates the filamentous growth during sexual differentiation via the Cwc1/Cwc2 complex. To further understand the regulatory mechanisms, we conducted a mutagenesis screen in the *CWC1* overexpression background to identify mutants restoring the mating filamentation under light conditions. An *Agrobacterium* T-DNA insertional mutant AY18 was found to restore mating filamentation and also dramatically de-repress the production of monokaryotic filaments. Further characterization revealed that disruption of a *Saccharomyces cerevisiae SSN8* homologue was responsible for the phenotypes. *C. neoformans SSN8* gene encodes a cyclin box containing Mediator protein, which serves as a transcriptional regulator in the eukaryotic cells. *S. cerevisiae* Mediator complex consists of 23 members, which are highly conserved from yeast to human. They function to connect other transcriptional regulators to the RNA polymerase ¢º, and help regulating many physiological processes positively or negatively at the transcriptional level. In *C. neoformans*, we found *SSN8* primarily functioned as a negative regulator in many physiological aspects as well. When the *SSN8* gene was deleted, sexual reproduction, both mating and monokaryotic fruiting, was enhanced. Besides, the *ssn8* mutant strains showed larger capsules and more melanin formation *in vitro*. Furthermore, the normal patterns of invasive growth and cell morphology were changed in the *ssn8* mutant strains. Based on our results, we conclude that the Mediator component Ssn8 plays negative roles in diverse physiological processes in *C. neoformans*.

141. Ndt80p genome-wide mapping provides insights into its role in sterol metabolism regulation and azole drug resistance in *Candida albicans*

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Candida albicans is a major cause of morbidity and mortality in bloodstream infections and is the most common fungal pathogen isolated from immuno-compromised patients. In addition to the limited drug targets, emergence of drug resistance in this pathogen reduced considerably the effective use of antifungal drugs. To better elucidate the role of the transcription factor Ndt80p in mediating azole tolerance, Chromatin Immuno-Precipitation-chip was used to determinate the genome-wide occupancy of this regulator. Ndt80p was found to bind a large number of gene promoters of diverse biological functions. Gene ontology analysis of Ndt80p bound promoters revealed a significant enrichment in genes related to multidrug responses (P = 2.09e-04) including drug transporters (*CDR1,2,4* and *MDR1*) and the azole target Erg11p as well other ergosterol biosynthesis genes. By combining genome-wide location and Microarray expression analysis, we have characterized for the first time the Ndt80p fluconazole-dependant regulon and elucidated the key role of this TF as an activator of ergosterol metabolism genes. Additional results of the potential interplay between Ndt80p and the sterol metabolism regulator Upc2p will be presented.

142. Regulation of gene expression by phosphate in *Neurospora crassa*

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Pi acquisition system in *Neurospora crassa* includes at least four regulatory genes: *nuc*-2, *preg*, *pgov* and *nuc*-1. Under limiting Pi conditions, NUC-2 inhibits the functioning of the PREG-PGOV complex, allowing the activation of the transcription factor NUC-1 and the expression of genes involved in phosphate acquisition. Aiming at a better comprehension of the *nuc*-2 functionality in gene expression regulation in response to exogenous Pi levels, two cDNA subtraction libraries were constructed comparing *N. crassa* wild type *St.L.*74A and *nuc*-2A strains, grown under Pi starvation. We obtained 52 NUC-2 up- and 16 down-regulated genes. Among the NUC-2 negatively modulated genes, we identified the MAK-2 (mitogen-activated protein kinase-2) protein coding gene, involved in intracellular signaling. The functional role of this gene in the extracellular Pi sensing was evaluated by microarrays, comparing wild type and *mak*-2 strains responses under Pi starvation. We identified 4214 MAK-2 regulated genes, among them the cyclin coding gene, *preg*. Furthermore, 3174 genes regulated in response to Pi levels were identified. The results obtained reveal novel molecular aspects of the adaptation to Pi availability, suggesting that *mak*-2 gene constitutes a novel component of the *N. crassa* phosphate sensing and signaling pathway.

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143. Mechanisms of action of transcriptional regulators involved in fatty acid catabolism.

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FarA, FarB and ScfA are Zn(II)2Cys6 transcription factors which upregulate genes required for growth on fatty acids in *Aspergillus nidulans*. FarA and FarB are highly conserved and bind to CCGAGG core sequences in the promoters of their target genes. My project focuses on investigating how they work together to control gene expression. *farA* overexpression strains show an increase in reporter gene activity in the presence of oleate but a decrease is seen when *farB* is overexpressed. This led to a proposed model in which a FarA homodimer activates genes when a long-chain fatty acid is present, but a FarA-FarB heterodimer (with ScfA potentially interacting) increases expression during growth on short-chain fatty acids. Protein interactions are being determined using yeast two hybrid analysis. In the heterodimer, FarB might receive the short-chain fatty acid signal, while FarA provides the activation ability. FarB-FarA fusion proteins were created in which different putative activation domains of FarA were inserted into the C-terminus of FarB, creating FarB proteins that have activation capability. These proteins were tested in a *farA?* background with growth significantly increased compared to wildtype *farB*. The activation ability of FarA may be its main contribution to short-chain fatty acid dependent expression, but it may also have other roles.

144. The Hat1 Histone Acetyltransferase Is Required for Maintenance of the Yeast Morphology in *Candida albicans*

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Candida albicans (Ca), the most prevalent human fungal pathogen, especially for immunocompromised patients, shows several distinct growth morphologies including yeast, pseudohyphal or true filamentous (i.e. hyphae) forms. Filamentation is considered a major virulence trait, as it facilitates systemic dissemination of *Ca* in the host. Under yeast-promoting conditions, the hyphal growth program is repressed by a set of dedicated transcriptional repressors, suggesting that relief of this repression is necessary for filamentous growth. To study the role of chromatin modifying enzymes in this process, we have investigated the role of the *Ca* orthologue of the *Saccharomyces cerevisiae* histone acetyltransferase 1 (*HAT1*). Therefore, we constructed homozygous *Ca* deletion strains lacking Hat1 and analyzed the impact on morphogenesis. Cultures of *hat1*Delta-/Delta- strains grown under yeast-promoting conditions in YPD at 30°C show a slow-growth phenotype, with cells of elongated and partially filamentous morphologies when compared to the wild type control strain. In addition, the deletions showed normal hyphal induction upon serum treatment at 37°C. Interestingly, *hat1*Delta-/Delta- cells formed wrinkled colonies on YPD plates, indicating constitutive filamentous growth under these conditions. Reintegration of *HAT1* completely restored the wild type phenotype and normal colony morphology. Reverse transcription polymerase chain reaction with RNA isolated from *hat1*Delta-/Delta- cells grown under yeast-promoting conditions showed a dramatic upregulation of hypha-specific genes such as *ECE1* and *HWP1*. In contrast, expression levels of known hyphal repressor genes such as *TUP1* or *NRG1* were similar in comparison to wild type cells. In addition, upregulation of *TUP1*-repressed genes *RBT2* and *SOD4* indicated that *TUP1*- mediated repression requires Hat1 activity. These data provide first evidence for an important role of the Hat1 chromatin modifier in controlling filamentation in *C. albicans*. We propose that Hat1 is a repressor of filamentous growth, and implicated in maintaining the yeast morphology. Hence, Hat1 could be an important modulator of virulence of *Candida albicans*.

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145. Cellular responses of *Phytophthora infestans* to cyclic lipopeptide surfactants produced by *Pseudomonas* species

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Oomycete pathogens cause devastating diseases on plants and animals and their control heavily depends on agrochemicals. With increasing concerns about adverse effects of agrochemicals on food safety and environment the development of novel, environmentally friendly control strategies, preferably based on natural products, is demanded. Cyclic lipopeptides (CLPs) produced by *Pseudomonas* species were discovered as a new class of natural compounds with strong activity against oomycetes including the late blight pathogen *Phytophthora infestans*. The *Pseudomonas fluorescens* CLP massetolide A (MassA), has zoosporicidal activity, induces systemic resistance and reduces late blight in tomato. To gain further insight in the modes of action of CLPs, effects on mycelial growth, sporangia formation, and zoospore behavior were investigated, as well as the involvement of G-proteins in sensitivity of *P. infestans* to MassA. In addition to zoospore lysis, MassA disturbed other developmental stages in the life cycle of *P. infestans*. G alpha gain-of-function mutants were less sensitive to MassA suggesting involvement of G-protein signaling in the response of *P. infestans* to this CLP. In order to reveal primary targets of CLPs we also monitored genome wide changes in gene expression. A distinct set of genes appeared to be up- or down-regulated after exposure to MassA, including genes encoding membrane transporters, alkaline phosphatases and pirins. Further characterization of these genes is in progress.

146. Is induction of trichothecenes B biosynthesis controlled by PAC in *Fusarium graminearum*?

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Tri genes responsible for the biosynthesis of *Fusarium* trichothecenes B mycotoxins are expressed *in planta* about four days after infection. *In vitro*, in GYEP or in a synthetic medium, the Tri genes of *F. graminearum* strain CBS 185.32 are expressed at day 3 to 4 with the toxin starting to accumulate one day latter. Strikingly, the induction of Tri genes expression always seems concomitant with a sharp pH drop in the media. Acidic pH seems determinant for induction as neither the toxin nor the Tri genes are detected at neutral pH. In addition, buffering the synthetic medium to neutrality after the pH drop reverses the induction of Tri genes and stops the toxin production. The PAC transcription factor involved in control of pH homeostasis in fungi was shown to regulate biosynthesis of various secondary metabolites. Here we studied effects of pH and the role of FgPAC on toxin accumulation and on Tri genes expression in *F. graminearum*. A deletion mutant and a strain expressing a constitutively active $FgPAC^{C}$ form were constructed. Deletion does not affect toxin production, but expression of the constitutive PAC^{C} factor strongly reduces expression of Tri genes and toxin accumulation. PAC consensus GCCARG fixation boxes are present in the promoters of various Tri genes. Whether or not FgPAC directly represses the expression of these genes by recognition of these boxes is now investigated.

147. Characterisation of serine proteinase expression in *Agaricus bisporus* and *Coprinopsis cinerea* using bioinformatics, GFP promoter analysis and gene silencing.

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The *Agaricus bisporus* serine proteinase 1 (SPR1) appears to be significant in both mycelial nutrition and senescence of the fruiting body. We report on the construction of an SPR promoter::GFP fusion cassette for the investigation of temporal and developmental expression of SPR1 in homobasidiomycetes, and to determine how expression is linked to physiological and environmental stimuli. Monitoring of *A. bisporus* SPR_GFP transformants on media rich in ammonia or containing different nitrogen sources, demonstrated that SPR1 is produced in response to available nitrogen. In *A. bisporus* fruiting bodies, GFP activity was localised to the stipe of postharvest senescing sporophores. SPR_GFP was also transformed into the model basidiomycete Coprinopsis cinerea. Endogenous *C. cinerea* proteinase activity was profiled during liquid culture and fruiting body development. Maximum activity was observed in the mature cap, while activity dropped during autolysis. Analysis of the *C. cinerea* genome revealed 7 genes showing significant homology to the *A. bisporus* SPR1 and SPR2 genes. These genes contain the aspartic acid, histidine and serine residues common to serine proteinases. Analysis of the promoter regions revealed at least one CreA and several AreA regulatory motifs in all sequences. Fruiting was induced in *C. cinerea* dikaryons and fluorescence determined in different developmental stages. GFP expression was observed throughout the life cycle, demonstrating that serine proteinase can be active in all stages of *C. cinerea* fruiting body development. Serine proteinase expression (GFP fluorescence) was most concentrated during development of young tissue, which may be indicative of high protein turnover during cell differentiation. To help further elucidate roles for SPR1 we chose the endogenous serine protease gene of *A. bisporus* as a target for gene silencing. Silenced lines have been generated and these show a range of biological effects depending on the degree of silencing. Data on these studies will be presented. Suppression of genes involved in these pathways will help to further elucidate the complex biochemical pathways involved in post-harvest degradation.

148. How does VeA effect secondary metabolism in *Fusarium fujikuroi*?

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Fusarium fujikuroi is known as a rice pathogen causing hyperelongation of stalks and leaves due to production of gibberellic acids (GAs). Besides GAs, *F. fujikuroi* may also synthesize other toxins like fumonisins, fusarin C and bikaverin. Although the clustered genes responsible for synthesis of these secondary metabolites are well characterized, our understanding of their regulatory mechanisms is incomplete. In this study, we identified and characterized the *F. fujikuroi veA* gene and examined its role as a global regulator of secondary metabolism. Comparison of wild type and *veA* deletion strains in pathogenicity assays revealed no hyperelongation of rice when infected with the deletion mutant, suggesting a down-regulation of GA genes. To identify more genes influenced by VeA we examined changes in gene expression of wild type and *veA* knock-out strains by use of a *F. verticillioides* oligo microarray. We found that among the down-regulated genes are those involved in sexual development as well as genes needed for GA and fumonisin synthesis. Up-regulated genes included those involved in bikaverin synthesis. No changes were found for genes involved in fusarin C production. Northern blot analysis confirmed the microarray data. Examination of *veA*-regulated genes led to the identification of a putative ortholog of *laeA*, a critical regulator of secondary metabolism in *Aspergillus*. The impact of this gene on *Fusarium* secondary metabolism is currently under investigation. Our results further prove the cross-species use of the *F. verticillioides* microarray to elucidate the diverse effects VeA has on secondary metabolism in *F. fujikuroi*.

149. The coordinated cellular response to nitrogen availability in *A. nidulans.*

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Perception of the quality and quantity of nitrogen available to the cell and the coordinated response in A. nidulans involves multiple regulatory and signalling mechanisms. Our current research is focused on three areas: The post-translational modification and intracellular localisation of AreA and other transcription factors involved in regulating nitrogen metabolism. The molecular mechanisms involved in regulating mRNA stability which for a large number of genes acts in tandem with transcriptional regulation to determine appropriate levels of gene expression in response to changes in nitrogen availability and quality. Global assessment of gene regulation, utilising proteomics, transcriptomics and high-throughput transcriptome sequencing. Our aim is to monitor the full complexity of the regulatory response to changes in the nitrogen regime. We shall present our recent data which highlights the interrelationships between nitrogen and other regulatory responses.

150. Role and regulation of the Nox family in the filamentous fungus *Podospora anserina*

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Studies on NADPH oxidases/Nox, a large family of enzymes dedicated to ROS production, have emphasized the biological importance of ROS in humans, plants and fungi. Three evolutionary distinct Nox isoforms are present in fungi, Nox1, Nox2 and Nox3. Studies on Nox1 and Nox2 in the coprophilous fungus *Podospora anserina* and in other ascomycetes have revealed the many important and pleiotropic functions of both enzymes in fungi. In the laboratory, we currently work on Crippled Growth (CG), an epigenetic cell degeneration syndrome, which proved to require Nox1. We now report the characterization of a new mutant defective in CG, *IDC*^{524}. The gene mutated in this strain codes for the NoxR/p67^{phox} regulatory subunit and we show that NoxR regulates both Nox1 and Nox2 in *P. anserina*. Unexpectedly, both *Nox1 Nox2* double mutant and *NoxR* mutant show drastic increase in ROS production highlighting alternative sources of ROS inhibited by Nox in fungi. Sequence analysis of *P. anserina* genome has identified the Nox3 isoform, raising the question whether Nox3 is the alternative source of ROS. With the aim to answer this question, we have generated the *Nox3* K.O mutant and we have compared phenotypes of all *Nox* mutants, alone and in combination . This work is funded by contract # ANR-05-BLAN-0385-01 from the ANR.

151. Analysis of the pheromone response-signaling cascade in *Ashbya gossypii.*

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A prerequisite for classical genetic analysis in a fungus is the knowledge of its mating system to generate meiotic offspring. The genome sequence of the filamentous ascomycete *Ashbya gossypii* revealed three mating-type loci on chromosomes IV, V, and VI, encoding two divergently transcribed genes, MATa1 and MATa2 each. Analysis of the genome sequence furthermore shows that all classical elements of the *Saccharomyces cerevisiae* mating-pheromone response pathway are present. Starting from pheromone receptor genes orthologous to *ScSTE2* and *ScSTE3*, to the heterotrimeric G-protein (*GPA1, STE4, STE18*), the MAP- kinase cascade (*STE11, STE7, FUS3/KSS1*) and the transcription factor *STE12*. Mating pheromone genes are annotated as homologs to *MFA2/YNL145w* (ABL196c) and *MFalpha1/YPL187w* (AFL062w). We re-sequenced AFL062w, however, this gene appears to be truncated and does not encode a mature alpha pheromone. On the other hand, AAR163c was found to encode one copy of an *A. gossypii* alpha factor. In the closest relative of *A. gossypii, Eremothecium cymbalariae*, orthologs of AFL062w and AAR163c both encode alpha factor peptides that are divergent in sequence to the *A. gossypii* alpha factor. We obtained the chemically synthesized *A. gossypii* alpha factor peptide. However, treating *A. gossypii* spores or mycelial fragments with this alpha-factor peptide did not elicit any visible response. To demonstrate functional pheromone-receptor interaction we expressed the *A. gossypii STE2* alpha factor receptor in a *S. cerevisiae ste2* mutant. Cells of such a strain arrest growth upon treatment with the *A. gossypii* alpha factor with a high sensitivity. Deletion of pheromone signal- transduction pathway genes, including *AgSTE2, AgSTE3*, and *AgSte12*, did not result in the inhibition of sporulation, suggesting that this pathway is not required for sporulation in *A. gossypii*.

152. Interaction of HapX-HapB/C/E complex with the cytochrome *c* promoter.

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The *Aspergillus* CCAAT-box binding complex consists of the HapB, HapC and HapE subunits. Previously we isolated HapX as a protein that interacts with the HapB/C/E complex[1)]. Recently, it was shown that various iron-dependent pathways are repressed under iron depleted conditions by interaction of HapX with the HapB/C/E complex[2)]. HapX harbors a basic domain from the b-Zip motif but a coiled- coil domain, instead of leucine-zipper, suggesting that HapX has DNA-binding ability, which is different from *Saccharomyces cerevisiae* Hap4p. In this study, therefore, we analyzed the interaction of the HapX-HapB/C/E complex with the promoter of the cytochrome *c* gene (*cycA*) of *Aspergillus nidulans*, which is one of the HapX-dependent genes. Electrophoretic mobility shift assays (EMSA) and DNA footprint analyses using recombinant HapX-HapB/C/E and various DNA probes demonstrated that both the CCAAT-box and an adjacent sequence of the *cycA* promoter are required for the recognition by the HapX-HapB/C/E complex. The DNA-binding ability of HapX presumably allows independent regulation by HapB/C/E and HapX-HapB/C/E. 1) Tanaka et al. B.B.A. 1576:176-182 (2002). 2) Hortschansky et al. EMBO J. 26: 3157-3168 (2007)

153. AreB – another player in the the nitrogen regulation network in *Fusarium fujikuroi*

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In *Fusarium fujikuroi*, the biosynthesis of gibberellins (GAs) and bikaverin, both nitrogen-free metabolites, is strictly inhibited by nitrogen. While GA biosynthetic genes are under control of AreA-mediated nitro¬gen metabolite repression, this transcription factor is not essential for expression of bikaverin genes. Thus, GA and bikaverin biosynthetic genes can be used as reporter genes for AreA-dependent and –independent nitrogen regulation. In contrast to *Aspergillus nidulans*, NMR as well as the bZIP transcription factor MeaB were shown to play only minor roles as antagonists of AreA activity: the expression of GA-and bikaverin biosynthesis genes was only slightly derepressed in the *meaB* and *nmr* deletion strains. Searching for more powerful inhibitors of AreA activity, we studied the role of the GATA transcription factor AreB by creating single *areB* and double *areA/areB* mutants. Surprisingly, *areB* mutants are not able to grow on glutamine, but grow very well on glutamate suggesting that AreB positively regulates glutamate synthetase. In contrast to *areA* mutants that are not able to activate nitrate reductase rendering them resistant to chlorate, deletion of *areB*resulted in high sensitivity to chlorate indicating that AreB indeed acts as negative counterpart of AreA. Comparison of expression pattern between wild-type and mutant strains revealed a strong down-regulation of GA-genes and up-regulation of bikaverin genes reminding of the situation in the *veA* mutants. Studies on cellular localization of AreB in response to nitrogen by fluorescent protein fusions are on the way. We will present a putative model of nitrogen regulation in *F. fujikuroi* .

154. Environmental regulation and transcript profiling of the reproductive phase change in *Agaricus bisporus*

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We have investigated the phase change from vegetative mycelium to reproductive growth in the white cultivated mushroom *Agaricus bisporus*. Environmental triggers are known to regulate fruiting in higher fungi and this knowledge has been used by the mushroom cultivation industry to control crop production. Temperature, carbon dioxide level and the concentration of 8-carbon volatiles are involved in the reproductive phase change in *A. bisporus*. However, it is not known how each stimulus affects the process at the molecular level and whether the control of fruiting is under a single or sequential regulatory mechanism. A suppression subtractive hybridisation and custom oligo-microarray screening approach was employed to investigate the transcriptional changes in *A. bisporus* to environmental and morphogenetic change. The initiation of fruiting under standard growing conditions was compared with experiments where temperature, carbon dioxide or volatile levels were not altered. The data has been used to profile the response of *A. bisporus* genes during fruiting and to identify the genetic response to individual stimuli. Evidence shows temperature affects later stages of mushroom development, while carbon dioxide and volatile levels regulate the initial switch from vegetative mycelium to reproductive phase.

155. Hypovirus-responsive transcription factor gene *pro1* of the chestnut blight fungus *Cryphonectria parasitica* is required for female fertility, conidiation and stable maintenance of hypovirus infection.

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We report characterization of the gene encoding putative transcription factor PRO1 identified in transcriptional profiling studies as being down-regulated in the chestnut blight fungus *Cryphonectria parasitica* in response to infection by virulence- attenuating hypoviruses. Sequence analysis confirmed that *pro1* encodes a $Zn(II)_2Cys_6$ binuclear cluster DNA binding protein with significant sequence similarity to the *pro1* gene product that controls fruiting body development in *Sordaria macrospora*. Targeted disruption of the *C. parasitica pro1* gene resulted in two phenotypic changes that also accompany hypovirus infection, a significant reduction in asexual sporulation that could be reversed by exposure to high light intensity and loss of female fertility. The *pro1* disruption mutant, however, retained full virulence. Although hypovirus CHV1-EP713 infection could be established in the *pro1* disruption mutant, infected colonies continually produced virus-free sectors, suggesting that PRO1 is required for stable maintenance of hypovirus infection. Deletion of the hypovirus ORF A coding domain, that encodes the p29 suppressor of RNA silencing, restored stable viral maintenance in the *pro1* disruption mutant stain. These results are discussed in terms of the role of host transcription factors in hypovirus persistence, transmission and elaboration of hypovirus-medated alterations of host phenotype.

156. Genetic characterization of *ust1*, an ortholog of *Aspergillus* StuA and potential master regulator of sporulation in *Ustilago maydis*.

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Ustilago maydis, the causal agent of corn smut is an important model system for biotrophic plant pathogenesis. During the course of its life cycle, *U. maydis* changes in morphology after mating from haploid yeast like cells to a dikaryotic filamentous form, which infects the host and produces pigmented diploid teliospores. The deletion of *ust1*, an ortholog of the *Aspergillus* developmental modifier, StuA, resulted in a mutant that reproduces these characters in culture. This project aims to study the gene network involved in teliospore development using *ust1* as a surrogate system. Thirty seven genes were identified to be down regulated in the filamentous cells in an earlier study. Of these 13 had putative *ust1* binding sites indicating the possibility of their regulation by it. To study the regulatory role of *ust1*, we are deleting 10 of these genes using the DelsGate technique. To date deletion constructs of all 10 genes have been made and 3 genes deleted in *U. maydis*. None of the 3 deletion strains showed any critical reduction in pathogenicity, but the deletion of the gene um11400 (probable thiamine biosynthetic enzyme) resulted in thiamine auxotrophy. A microarray analysis comparing the *ust1* mutant to wild type was performed to identify the effect of *ust1* deletion on the global gene expression. Differential expression of these 13 genes indicate the possibility that *ust1* may act both as an activator and repressor. To identify other components of sporulation pathway and their interaction with *ust1*, we generated approximately 100 suppressor mutants by UV irradiation and classified them into 10 phenotypic groups. Complementation of these suppressor mutants using a genomic DNA library is being pursued.

157. *Candida albicans* self-control: Novel regulation of Ras1 signaling

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The reversible transition between yeast and hyphal growth is a key virulence factor in the human fungal pathogen, *Candida albicans*, and it is clear that complex regulation governs this morphogenesis. In addition to sensing and responding to exogenous cues provided by the environment, *C. albicans* regulates its own morphology by the production and secretion of several factors, including dodecanol and the autoregulatory molecule, farnesol. Previous work from our lab has demonstrated that these secreted molecules prevent the yeast to hypha transition by disrupting signal transduction through the Ras1-Adenylate Cyclase(AC)-Protein Kinase A(PKA) pathway upstream of PKA activation (Davis-Hanna *et al.,* 2008). Western blot analysis of Ras1p from wild- type *C. albicans* cells cultured under conditions that promote hyphal growth reveals an increase in levels. Furthermore, a high molecular weight Ras1p (HMWRas1p) species accumulates throughout hyphal growth, with levels decreasing as hyphal cultures revert back to yeast form growth. Activation of Ras1p is not required for the formation of HMWRas1p. Strains in which cAMP signaling is disrupted due to deletions of AC(*Delta-cyr1/Delta-cyr1*) or the cyclase associated protein, Srv2(*Delta-srv2/Delta-srv2*), do not form hyphae but accumulate HMWRas1p, indicating that its formation is not dependent upon hyphal growth or downstream signaling events. We are currently investigating how this modification contributes to hyphal growth and its repression by secreted signaling molecules.

158. Nuclear dynamics during *Cryptococcus neoformans* mating and monokaryotic fruiting

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Disseminated basidiospores are considered a major infectious propagule for cryptococcal infection. *Cryptococcus neoformans* undergoes two distinguishable sexual cycles, mating and monokaryotic fruiting, to produce basidiospores. In mating, two different mating type cells fuse (plasmogamy) to form hyphae in which two haploid nuclei exist as a dikaryon. After formation of basidia, the nuclei undergo nuclear fusion (karyogamy) to form a diploid followed by a meiosis to produce haploid progeny. In monokaryotic fruiting, diploidization occurs to form diploid nuclear during filamentation and the diploid nuclei undergo meiosis. The roles of karyogamy genes and proteins in these developmental stages are poorly understood. In this study, we identified five karyogamy (*KAR*) gene orthologs in *C. neoformans* genome by a blastp search with *Saccharomyces cerevisiae KAR* genes. *CnKAR3* and *CnKAR4* encode proteins involved in nuclear migration and *CnKAR2*, *CnKAR7*, and *CnKAR8* gene products are involved in nuclear membrane fusion. There are no apparent orthologs of *ScKAR1* or *ScKAR5*. *kar7* mutants display significant defects in hyphal growth and basidiospore formation during monokaryotic fruiting. In an a *kar7* x alpha *kar7* bilateral mutant cross, we also observed a delay in hyphal growth and defective basidiospore formation. These results demonstrate that the *KAR7* gene plays a critical role in mating and monokaryotic fruiting, implying proper control of nuclear dynamics maybe important. Further studies on nuclear localization of Kar7, expression of *KAR* genes, and roles of the other *KAR* genes during mating and fruiting, are in progress.

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159. Ancestral homologs of the yeast bud site selection proteins regulate septum formation and development in filamentous fungi.
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The defining feature of fungal cells is polarized growth, whereby cell wall deposition is confined to a discrete location on the cell surface. The annotation of multiple fungal genome sequences has revealed that the signaling modules and morphogenetic machinery involved in polarized growth are largely conserved across the fungal kingdom. Nevertheless, fungal cells exhibit a diverse variety of shapes that are largely based on two growth patterns: hyphae and yeast. We suggest that these different patterns reflect variation in the mechanisms that spatially and temporally regulate cellular morphogenesis. To test our hypothesis, we are characterizing ancestral homologues of the yeast bud site selection proteins. In particular, we have found that the yeast axial bud pattern markers Bud3, Bud4, and Axl2 are weakly conserved in the *Pezizomycotina*. Functional studies in *Aspergillus nidulans* implicate Bud3 and Bud4 in the regulation of septum formation and suggest that Bud3 may serve as a guanine nucleotide exchange factor for the GTPase Rho4. By contrast, Axl2 is dispensable for hyphal morphogenesis, but is required for cytokinesis of spores from phialides during conidiation. Moreover, Axl2 also appears to repress sexual development in a light- and VeA-independent manner. Additional genetic analyses suggest that Axl2 functions upstream of the GTPase Cdc42 in a pathway that regulates the timing of sexual development. Because loss of the *Fusarium graminearum* Axl2 homologue also triggers precocious sexual development, we propose that this may represent an ancestral pathway that coordinates the development of ascomycete fruiting structures.
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160. Unraveling transcriptional regulatory mechanisms involved in sexual reproductio of *Phytophthora infestans*.
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Phytophthora infestans, the causal agent of potato and tomato late blight, is one of the most devastating plant pathogens in the world. Its sexual reproduction cycle serves an important role in disease since the sexual spores (oospores) are important for survival over the long-term and through unfavorable conditions. To understand the transcriptional regulatory machinery involved in sexual development, ten genes that based on microarray studies are expressed specifically during mating, or up-regulated more than 100-fold, were selected for detailed analysis by fusing their promoters with the GUS reporter gene, and transformed into *P. infestans* to examine their temporal and spatial expression patterns. The promoter of gene Pi 000192, which is predicted to encode an elicitin-like protein, has shown specific activity in oogonia and antheridia. Deletions, site-directed mutagenesis, and electrophoresis mobility gel shift assays are now being used to define the regulatory elements in this promoter. To acquire further information on the sexual stage, a global proteomics comparison of the vegetative (nonsporulating mycelium) and sexual (oospore) life stages of *P. infestans* is being conducted. A preliminary study indicated that oospores contain mitochondrial proteins, ATPase, crinkler family proteins, and others. In addition, the functions of selected mating-induced proteins are being tested, such as a RNA-binding protein belonging to the Puf family. Tandem affinity purification tags were attached to the Puf RNA binding domain to use affinity approach to identify mRNAs that bind and are presumably regulated by Puf during sexual development.

161. Knock-out of the global nitrogen regulator AreA in *Fusarium graminearum* .
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AreA is a global regulator of nitrogen metabolism in many fungi including *Fusarium sp*. The production of gibberellin in *Gibberella fujikuroi* (Tudzynski et al. 1999) and fumonisin B1 in *Fusarium verticillioides* (Kim et al. 2008) has been shown to be regulated by AreA. *Fusarium graminearum* is a serious pathogen of wheat and barley and is infamous for producing a variety of toxins such as DON, NIV and ZEA in the grain. To determine the role of AreA in the regulation of secondary metabolism in *Fusarium graminearum* the gene was replaced in PH-1 by *Agrobacterium tumefaciens* mediated transformation. The AreA KO phenotype shows reduced radial growth and production of arial mycelium. The strain can utilize the preferable nitrogen sources such asparagine and ammonium and have a tolerance to chlorate when grown on rich medium. Growth on medium containing nitrate is inhibited. Ongoing experiments are aimed at determining the ability of the strain to produce specific toxins on different media. LC/MS/MS analyses will be used and comparisons to the WT cultivated under the same conditions will be carried out. Complementation of the AreA using geneticin as selection marker will be performed to gain full recovery and utilize nitrate as nitrogen source as well as produce of DON, NIV and ZEA.

162. Two different RNA-dependent RNA polymerases involved in induction and amplification of RNA silencing in *Mucor circinelloides*.
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The silencing mechanism in the zygomycete *Mucor circinelloides* is associated with two size classes of siRNA, 25 and 21-nt long. These two siRNAs are preferentially produced from the 3'-end of the target genes and are associated with amplification of the silencing signal. Induction of silencing by non-integrative sense transgenes requires an RNA-dependent RNA polymerase (RdRP) to convert aberrant RNA transcripts into dsRNA molecules. Silencing amplification also requires an RdRP enzyme for production of secondary siRNAs. We have cloned two *Mucor rdrp* genes and have obtained null mutants by gene disruption. To investigate the role of these RdRP enzymes in the induction and amplification of silencing, we have compared the efficiency and stability of gene silencing triggered by sense transgenes with that obtained using hairpin RNA expressing constructs as triggers, both in the wild type and in mutants affected in the *rdrp-1* or *rdrp-2* genes. Results indicate that *rdrp-1* gene is essential for induction of silencing by sense transgenes, but it is not involved in the amplification of silencing induced by dsRNA. Coversely, the *rdrp-2* gene is not involved in the induction of silencing but it is required for efficient amplification of the silencing signal. These results indicate a kind of specialization of the RNAi machinery in different pathways of the silencing mechanism.

163. Profiling of gene expression during polar growth of *Aspergillus fumigatus*

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A. fumigatus is the most common airborne pathogen causing fatal mycoses in immunocompromised patients. Polarized growth is one of the critical factors for establishing fungal pathogenesis, but little is known about the genes involved in early polar growth and their regulation. The purpose of this study was to find polar growth related genes in *A. fumigatus*. *A. fumigatus* Af293 was cultured in complete medium and total RNA was extracted at set time points. DNA microarray experiments were performed comparing dormant cells (0hr) with isotropicly growing cells (4hr), isotropicly growing cells with cells showing emerging germtubes (6hr), and with more mature hyphae (8hr). Expression of selected genes was confirmed by RT-PCR. Comparison of altered genes in the three array experiments shows that the change of gene expression is most prominent at the switch from dormant to isotropic growth and that many genes show significant expression changes during early growth in *A. fumigatus*.

164. Identification of Mat2 and Znf2 as central elements of the circuit governing sexual development of *Cryptococcus neoformans*

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Cryptococcus neoformans is a human fungal pathogen that undergoes a dimorphic transition from a unicellular yeast form to a multicellular hyphal form during opposite sex (mating) and unisexual reproduction (monokaryotic fruiting). A conserved pheromone sensing Cpk1 MAPK signal transduction cascade governs the dimorphic switch in *C. neoformans* during both mating and fruiting, similar to pathways that operate during mating in other fungi. Mating and fruiting are induced by similar environmental conditions, and involve many shared components. However, the homeodomain cell identity protein Sxi1alpha that is essential for completion of sexual reproduction following cell-cell fusion during opposite sex mating is dispensable for fruiting. Therefore, identification of downstream targets of the Cpk1 MAPK pathway holds the key to the understanding of the molecular mechanisms governing the two distinct developmental fates. Thus far, homology-based approaches failed to identify downstream transcription factors which may therefore be species-specific. Here, we applied random insertional mutagenesis via *Agrobacterium*-mediated transformation and transcription analysis using whole genome microarrays to identify factors involved in *C. neoformans* differentiation. Two transcription factors, Mat2 and Znf2, located in regions outside of the *MAT* locus, were identified as key regulators of hyphal growth during mating and fruiting and . Genetic, phenotypic, and transcriptional analyses of Mat2 and Znf2 provide evidence that Mat2 is a downstream transcription factor of the Cpk1 MAPK pathway whereas Znf2 functions as a more terminal fate determinant. Further characterization of these elements and their target circuits will reveal genes controlling biological processes central to fungal development.

165. A novel approach for discovering motifs regulating spore-specific transcription in the phytopathogen *Phytophthora infestans*

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The oomycete *Phytophthora infestans* is one of the most devastating phytopathogens, causing late blight in potato and tomato. Its pathogenic success depends on forming different asexual spores such as sporangia and zoospores. Our goal is to identify what regulates transitions between such stages, by understanding what determines stage-specific transcription. To help accomplish this, transcription factor binding sites (TFBSs) acting during development are being identified by integrating bioinformatics and traditional molecular biology techniques. Microarray data was employed to help assemble sets of stage-specific and coexpressed promoters. These were searched for over-represented motifs (putative TFBSs) using Gibbs sampling, enumerative search, and expectation maximization algorithms. Phylogenetic footprinting involving eight *Phytophthora* genomes, and tests for positional bias, are being used to provide robust TFBS predictions. So far, data has been obtained from genes co- expressed in sporangia and cleaving sporangia. For each, multiple putative TFBSs were identified. Known motifs, such as the "cold box" which regulates some zoosporogenesis-induced genes, were in the output; this supports the validity of the computational approach. In addition, many motifs show evolutionary conservation and positional bias, suggesting that they are biologically relevant. Functional analyses of selected motifs are showing encouraging results, confirming that integrating bioinformatics with traditional promoter analysis methods reduces the time needed to identify TFBSs. This should lead to a better understanding of signaling pathways regulating spore development and provide insight into new disease control strategies.

166. Interaction of LaeA with NsdC, a C_2H_2-transcription factor involved in fungal development

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LaeA has been shown to be a master regulator of transcription of genes in some secondary metabolite gene clusters in Aspergilli. How LaeA functions is still unclear although recently LaeA was shown to bind to VeA, the protein involved in light sensing during asexual development in *A. nidulans*. Using a yeast two-hybrid detection system we now report that LaeA binds to NsdC, a C_2H_2- type transcription factor required for sexual development in Aspergilli. For the analysis, vectors were constructed where *A. parasiticus laeA* was used as the bait and cDNA from mRNA from 48 h *A. parasiticus* grown under conditions that induce aflatoxin production was used as the prey. One hundred eighteen clones were obtained on selective medium using a Clontech Matchmaker Kit. Of the clones examined two showed interaction with LaeA by co-immunoprecipitation. One of these was identified as a homolog to an *Aspergillus clavatus* C_2H_2 zinc- finger protein transcription factor (XM_001273230.1). Protein BLAST search of the translated fungal gene database revealed that this protein was encoded by a homolog to *nsdC*, a gene in one of four complementation groups from A. nidulans mutants unable to form sexual structures. The function of *nsdC* in sexual development has not been determined, but our results suggest that NsdC binding to LaeA may mediate expression of critical genes required for secondary metabolism and development.

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167. Histone modifiers, LSD1 and Bre2 homologs in *Aspergillus fumigatus*

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Histone modifiers have been shown to regulate gene expression through their ability to remodel chromatin structure. These epigenetic modifications are conserved in eukaryotic systems and have been well studied in yeast systems. Additionally, a handful of studies have reported the importance of chromatin remodeling in regulation of secondary metabolite gene clusters in the model organism *Aspergillus nidulans*. Here we set out to investigate the involvement of two chromatin remodeling enzymes in the pathogenic mold *A. fumigatus*, LSD1 (histone 3 lysine 4 demethylase) and BRE2 (COMPASS complex member – histone 3 lysine 4 methyltransferase) in secondary metabolism and virulence.

168. Posttranscriptional regulation of the Neurospora circadian clock

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The eukaryotic circadian oscillators consist of autoregulatory transcription-based negative feedback loops. However, the role of post-transcriptional regulation in circadian oscillators is unclear. In the Neurospora circadian clock, FREQUNENCY (FRQ) and a FRQ-interacting RNA helicase (FRH), form the FFC complex that represses the transcription of frq to close the circadian negative feedback loop. Here we show that, in addition to its role in transcriptional repression, FFC binds frq RNA and interacts with the exosome and to post-transcriptionally regulate frq decay. Consequently, frq RNA is robustly rhythmic as frq is more stable when FRQ levels are low and less stable when FRQ levels are low. Knock-down of RRP44, the catalytic subunit of the exosome, elevates frq RNA levels and impairs clock function. In addition, rrp44 is a clock-controlled gene and a direct target of the WHITE COLLAR complex. Our results further show that RRP44 is required for the circadian expression of a subset of ccgs. Taken together, these results suggest that FFC and the exosome are part of the post-transcriptional circadian negative feedback loop regulating frq levels. And as the machinery for mRNA degradation, exosome defines a novel post-transcriptional negative feedback loop in circadian system.

169. Establishment of RNAi method in *Lentinula edodes*.

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RNA-mediated gene silencing (RNA interference; RNAi) is a posttranscriptional gene- silencing phenomenon in which double-strand RNA (dsRNA) triggers degradation of cognate mRNA in a sequence-specific manner. RNAi has already been used for gene downregulation in plants, insects, animal and several fungi. In this study, we tried to apply RNAi in *Lentinula edodes* (white rot fungus), which is one of the most important edible mushroom in Japan. Laccase gene (*lcc1*) of *L. edodes* was used for target gene. Lcc1 is one of the lignin-degrading enzymes and secreted abundantly into liquid medium from *L. edodes* mycelium. We constructed a vector which expressed total 147 bp hairpin dsRNA of *lcc1*. The vector was transformed into *L. edodes*, and 57 transformants were obtained. Downregulation of the *lcc1* was observed in two transformants at transcriptional and translational levels. The *lcc1* transcriptional levels in these two transformants were downregulated at 0.2-1.7% compared to the wild type strain. Western blot analysis showed that expressions of Lcc1 were reduced in these two transformants. These results suggest that target gene could be downregulated by RNAi in *L. edodes*. And we also found that the *lcc1* downregulated transformants could not form fruiting body. This result suggests that Lcc1 might be involved in fruiting body induction in *L. edodes*. Downregulation by RNAi will be very powerful tool to analyze function of target gene in *L. edodes*.

170. Pde or not Pde? That is the question.

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Particularly important for the dimorphic switch between budding and filamentous growth and for pathogenicity, the activity of Protein Kinase A (PKA) depends on cAMP levels. Although the components of cAMP signaling are conserved from fungi to humans and many have been elucidated, we would like to further characterize components of this pathway in *Ustilago maydis*, a pathogen of maize. Phosphodiesterases (PDEs) are enzymes that degrade cAMP and thus help regulate the cAMP-PKA signaling pathway. After inspection of the *U. maydis* genome, we identified and selected three predicted homologues of PDEs to test the hypothesis that PDEs would be involved in cAMP turnover in this organism. If so, they would be expected to regulate activity of the PKA catalytic subunit and filamentous growth. The *pde1* gene is predicted to encode a low affinity cAMP PDE, whereas Pde2 and Pde3 contain InterPro domains associated with PDE activity. While neither disruption nor over-expression of these genes produced obvious phenotypes on rich agar, further investigation did reveal interesting differences. Mutants deleted for *pde1* displayed increased sensitivity to hyperosmotic conditions and both *pde1* and *pde3* deletion mutants displayed increased resistance to calcoflour compared to wild type. Cell morphology of the *pde1* deletion mutant resembled the multiple budding phenotype seen with *ubc1* mutants, although only about 30% of cells in a field showed this phenotype. The *pde1* deletion mutant also had a slight reduction in mating efficiency. To begin biochemical characterization of Pde1 and Pde2, we expressed both proteins in *E. coli*. Pde1 was successfully expressed only after eliminating an N-terminus membrane-spanning domain while Pde2 was expressed from full length sequence. Preliminary enzymatic analyses demonstrated that both Pde1 and Pde2 have phosphodiesterase activity *in vitro* and we are currently further purifying all three enzymes to facilitate biochemical and kinetic analysis of the proteins. These results demonstrate that Pde1 is a *bona fide* phosphodiesterase, however, Pde2 may be an AMP phosphatase and/or cAMP phosphodiesterase. Pde1 likely has important roles in cell morphology and development.

171. Human fungal pathogen *Cryptococcus neoformans* adapts to azole drug-stress by step-wise duplication of multiple chromosomes

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Cryptococcosis is an AIDS defining opportunistic fungal disease caused by the environmental yeast, *Cryptococcus neoformans*. Fluconazole, an azole drug, is widely used for the treatment of cryptococcosis due to its efficacy and safety. We previously described that *C. neoformans* strains are innately heteroresistant to fluconazole. Each strain yields minor subpopulation that can tolerate various concentrations of fluconazole ranging from 4μg to 64μg/ml without previous exposure to the drug. The heteroresistant subpopulations gain resistance in step-wise manner and revert to the original phenotype during the maintenance in the absence of the drug. We employed microarray and comparative genome hybridization arrays to study the difference between the subpopulation highly resistant to fluconazole and the wild type, H99. We found that the acquisition of resistance to higher concentrations of fluconazole in heteroresistant subpopulation is directly associated with duplication of multiple chromosomes. Upon repeated transfer in a drug-free media, this subpopulation with aneuploidy in multiple chromosomes loses the duplicated chromosomes in step-wise manner and return to original level of drug tolerance. Chromosomal duplication associated with increase in fluconazole resistance was also observed in other serotype A as well as serotype D strains. While this study may offer an explanation for the cryptococcosis relapse cases during the azole maintenance therapy, mechanism(s) of chromosomal duplications and genes that are possibly involved in the process need to be elucidated.

172. Analisis of *dicer* mutants of the filamentous fungus *Trichoderma atroviride* and its endogenous small RNAs.

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Trichoderma atroviride is used as model for light induced conidiation; it also produces conidia in response to mechanical injury, and is one of the most commonly used biocontrol agents. The molecular bases of conidiation are still poorly understood. An area that has not been explored is the possible participation of small RNAs in this process, and therefore the proteins involved in their biogenesis. In the sRNAs group, there are two categories, microRNAs (miRNAs) and short interfering RNAs (siRNAs), which main difference is the nature of their precursors. Dicer, RNA dependent RNA polymerase (RdRP) and Argonaute are key enzymes involved in the biogenesis and function of these sRNA. A wide range of fungi possess multiple components of the machinery to produce small RNAs in their genome, the participation of those components has been proven on post-transcriptional silencing by RNAi for some of those species, but there is scarce evidence of their involvement in the development and physiology of fungi. We have obtained single and double mutants of the *T. atroviride dicer* homologues. The mutants show growth rate and hyphal structure alterations. In addition, preliminary analyses suggest that biocontrol activity could be affected in the *dcr2* deletion mutant. In a sRNA library, generated from *T.atroviride* grown under different conditions, we found putative endogenous sRNAs that come from intergenic regions, or correspond to antisense transcripts of exons. These data suggest the possible participation of sRNAs in the development of this fungus.

173. The functional analysis of a new transcription factor gene identified by large-scale chromosome deletion in *Aspergillus oryzae*.

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In our previous report, we have constructed a mutant (?204-232) producing dense conidia by the large-scale chromosomal deletion in *A. oryzae*. In this study, to identify the gene responsible for the ?204-232 mutant phenotype, we conducted a series of deletions of 28 genes between AO090011000204 and AO090011000232. The deletion analysis indicated that AO090011000215(215) gene-disruption directly caused the similar phenotype to ?204-232 mutant. Moreover, hardly any sclerotium-like body is observed in the 215 gene-disruptant, whereas the parental strain normally produces the sclerotium when streak-cultured on the malt agar medium. BLAST analysis indicated that the 215 gene encodes a protein containing helix-loop-helix (HLH) motif, suggesting that it belongs to bHLH family of transcription factors. We then constructed 215 over-expression strain with *amyB* promoter, finding that this strain produces less conidia and more sclerotium-like body on the malt medium, in contrast with ?215 mutant. When these mutants were grown in DPY liquid medium, protein degradation and cell lysis were observed in D215 mutant after 3-day cultivation. These results suggest that the 215 gene plays an important role in retaining mycelial morphogenesis and the normal cell function in at least liquid medium. More detailed analysis and characterization of the functions of 215 are in progress.

174. Dual promoter vectors for high-throughput gene function analysis by RNA silencing.

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RNA silencing offers potent and flexible tools to explore gene function in the post- genomics era. We previously showed that the RNA-silencing vector, pSilent-Dual1 (pSD1) carrying two convergent promoters, *Aspergillus nidulans* TrpC (PtrpC) and gpd (Pgpd), was applicable for large scale loss of functional analysis in *Magnaporthe oryzae* (Quoc *et al.*, 2008). In this study, we constructed a new RNA silencing vector pSilent-Dual2 (pSD2) with two convergent TrpC promoters, and assessed the efficacy of RNA silencing induced by the dual promoter vectors in detail. The size of a target gene inserted into the vectors greatly affected the efficacy of gene silencing. In general, a smaller fragment induced gene silencing more efficiently either with pSD1 or pSD2 in *M. oryzae* even though a minimum of a 0.2kb fragment was necessary for inducing stable silencing. The pSD2 vector showed a little higher silencing efficacy than did pSD1 especially when insert size was relatively small. Correlation of gene silencing between transcriptionally-fused genes in pSD1 was examined using the xylanase (MG01542) or calcium channel (MGG05643) gene fused to an eGFP fragment. Analyses of enzymatic activity and quantitative PCR revealed that silencing of the target genes was moderately correlated (R=0.6-0.7) with that of the eGFP gene at the transcriptional and phenotypic levels.

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175. Analysis of novel conidiation regulatory genes in *Aspergillus oryzae* using omics approaches.

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Conidia of *Aspergillus oryzae* are used as starter of Japanese food industries. Therefore, study of conidiation regulatory pathway in *A. oryzae* will provide important information to improve the productivity of conidia, and we have analyzed conidiation regulatory pathway in *A. oryzae*. Previously, we found 7 kinds of novel conidiation regulatory genes from a transcriptional regulatory gene disruptants library in *A. oryzae*[1)]. To obtain further information of these genes, omics analysis of the wild-type strain and conidiation regulatory gene disruptants were carried out. Firstly, transcriptome analysis during conidiation in the wild-type strain was performed. Typical conidiation regulatory genes, such as *brl*A and *aba*A orthologs, were induced in conidiation period. Then, all of the 7 novel conidiation regulatory genes were constitutively expressed. Secondly, transcriptome and metabolome analysis of conidiation regulatory gene disruptants were carried out. These omics analysis data indicate that positions of these 7 novel conidiation regulatory genes are upstream of *brl*A ortholog in the conidiation regulatory pathway. Furthermore, we will report the statistical analysis data of these omics analysis data.

1) M. Ogawa, *et al*, (2008) 9th European Conference on Fungal Genetics, PR8.35.

176. Aspergillus nidulans multi drug resistance genes expressed in response to inhibitor agents

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The progressive resistance to drugs in fungi has brought serious economic and social consequences. This phenomenon is due to two main factors: the misuse of antifungals and limited number of mechanisms of action. In this context, the role of natural selection is stronger, which contributes to the increase in fungal resistance. Aspergillus nidulans has been a major model system, leading to the discovery of several key cellular processes related to resistance to drugs, among which is efflux of cytotoxic chemical compounds through ABC transporters (ATP -Binding-cassette). The study of these proteins has become important to elucidate the mechanisms of resistance to multiple drugs (MDR) and identify their possible involvement in pathogenic fungi. A cDNA clone library constructed with RNAs extracted from fungus grown in the presence of a large number of antifungal drugs was subtracted with radioactively labeled cDNA form fungus grown on glucose. Many differentially expressed genes were identified and they belong to stress metabolism and four cellular efflux pumps. The main feature of these genes, MDR-01, MDR-02, MDR-03 and MDR-04, are similarity to ABC transporters. Northern blots were done using these MDR genes as probes hybridized agains RNA extracted from fungus exposed to different inhibitors (acriflavin, undecanoic acid, amphotericin B, ethidium bromide, ketoconazole, fluconazole, griseofulvin, terbinafine and nystatin). It was possible to identify differential expression of these genes in the presence of several compounds chemically unrelated, suggesting that carriers must participate in a mechanism of resistance to multiple drugs. As the ABC transporters, in addition to the outflow of drugs, they may also secrete proteins involved in pathogenicity, potential targets for drug development.

177. A light regulation complex controls germination and further development in *Aspergillus nidulans*

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It was discovered recently, that plant-like phytochrome is involved in light sensing in the filamentous fungus *Aspergillus nidulans*. We could show that phytochrome (FphA) is part of a protein complex containing the white-collar homologues LreA and LreB, the two central components of the *Neurospora crassa* blue-light sensing system. We found that FphA represses sexual development and mycotoxin formation, whereas LreA and LreB stimulate both. Surprisingly, FphA also interacted with VeA, another regulator involved in light sensing and mycotoxin biosynthesis. According to the developmental effects we asked whether there are phase specific transcriptions factors that are part of the light regulation complex (LRC) or interact with it. We tested a number of candidate proteins and found that NosA (=number of sexual spores) and NsdD (=never sexual development) are part of the LRC. There are good reasons to believe that there is a phosphotransfer from FphA to NosA. Corresponding assays are in progress. Moreover we found that light controls the development of *A. nidulans* from the very beginning of it's lifecycle, with the germination of conidiospores. The formation of germ tubes is delayed upon illumination, especially with far red light. This effect depends on the presence of FphA but it is independent of the blue light sensing system.

178. Inducible RNA Interference in *Aspergillus nidulans*.

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RNAi is a eukaryotic mechanism in which small RNA molecules regulate gene expression and is used as a tool to silence expression of targeted genes. We have used *Aspergillus nidulans*, a multicellular fungus, to test an alcohol-dependent inducible construct for RNAi. The construct consists of inverted repeats of an alcohol dehydrogenase promoter, *alcA(p)*, with a gene of interest located in a unique restriction enzyme site between the promoters. Our gene of interest for silencing was *brlAbeta*, the longer of a two-transcript, differentially expressed gene that regulates asexual development. The RNAi strains show normal phenotypes on standard media containing glucose, but a remarkable loss of development on *alcA(p)* inducing media containing threonine, similar to that seen of *brlA* knockout mutants. Expression and lack of expression of *brlA* in the two respective growth conditions was confirmed with Northern blotting, RT-PCR, and Real Time RT-PCR. Our results confirm the RNAi construct induces silencing of a targeted gene. Although the *brlAbeta* transcript was targeted for RNAi, both *brlA* transcripts were absent on RNAi inducing conditions. Anti-sense and siRNA Northerns confirmed that the lack of *brlAalpha* expression was not due to RNAi mechanisms, but rather a result of reduced *brlAbeta* expression. We have replaced the *brlAbeta* gene in the RNAi construct with a library of genomic DNA and are characterizing phenotypes of the RNAi strains, with special attention to fungicide resistant strains.

179. Regulation of photocarotenogenesis via proteolysis-independent ubiquitylation in the zygomycete *Mucor circinelloides*

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Protein ubiquitylation plays a major role in the regulation of cellular processes through proteasome-dependent degradation of proteins, although it has become increasingly clear that it is also involved in mechanisms other than simple targeting to the proteasome. In *Mucor*, blue light regulates carotene biosynthesis. This response is controlled by two independent regulatory pathways, with *crgA* and *mcwc- 1c* as the corresponding key genes. CrgA shows characteristics of ubiquitin ligases and represses carotenogenesis, whereas *mcwc-1c* is a *white collar 1*-like gene required for light induction. The function of *crgA* in carotenogenesis is mediated by *mcwc-1b*, another *white collar 1*-like gene that acts as a carotenogenesis activator. CrgA controls MCWC-1b function by proteolysis-independent mono- and di-ubiquitylation, which results in MCWC-1b inactivation. This is the first description of this regulatory mechanism in filamentous fungi, and suggests that it could be more widespread than previously thought. A proteomic analysis revealed that CrgA regulates the levels of a number of proteins both through MCWC-1b and independently of it, suggesting that MCWC-1b is not the sole CrgA target. Moreover, these results suggest that proteolysis-independent ubiquitylation is used in *Mucor* to control other cellular processes, in addition to carotenogenesis.

180. Conservation of *ctg1* (the target gene for Le.CDC5) in basidiomycetous mushrooms, and their involvement in fruiting-body morphogenesis of *Lentinula edodes* and *Coprinopsis cinerea*.

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We have previously isolated the *ctg1* gene as a target for *Lentinula edodes* CDC5 (c-Myb-type DNA-binding protein Le.CDC5). It binds to a 7-bp sequence 5'GCAATCT3' in the transcribed region downstream of the start codon of *ctg1* and its interacting partner CIPB to 6-bp sequences in the 5'-upstream and transcribed regions of it. *ctg1* transcription appeared to be cooperatively regulated by Le.CDC5 and CIPB. We found basidiomycetous mushrooms *Coprinopsis cinerea* and *Laccaria bicolor* may also possess the gene-expression regulation system of *ctg1*–CDC5–CIPB. In *C. cinerea*, similarly to the case in *L. edodes*, the Le.CDC5 homologue (named Cc.Cdc5) was shown to bind to a 7-bp sequence 5'GCAAGCT3' in the transcribed region of the *ctg1* homologue (*Cc.ctg1*) and the CIPB homologue (Cc.CipB) to 6-bp sequences in the 5'- upstream and transcribed regions of it. To study the biological function of the *Cc.ctg1* (and *ctg1*) in *C. cinerea*, we bred the *C. cinerea* homokaryotic fruiting strains constantly producing a large amount of the *Cc.ctg1* (or *ctg1*) transcript and observed their phenotypes during fruiting development. It was found that stipe elongation in the strains constitutively expressing *Cc.ctg1* (or *ctg1*) starts earlier than in the control.

181. Mating Type Specific Signaling in *Ustilago maydis*.

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The phytopathogenic fungus *U. maydis* undergoes a dimorphic transition in which cell fusion and pathogenic development must occur for *U. maydis* to complete its lifecycle. Both cell fusion and pathogenicity are controlled by two loci, the *a* locus, encoding a pheromone and pheromone receptor, and the *b* locus, controlling pathogenic development. Mating of two cells of opposite mating type requires activation of the *a* locus via signal transduction through the mitogen-activated protein kinase (MAPK) pathway. The PAK-like Ste20p homologue, Smu1p, is required for a normal response to pheromone via up-regulation of *mfa* expression. Deleting *smu1* reduced this up-regulation of *mfa* expression, with the effect more pronounced in the a2 mating background. A similar mating type specific defect also occurs with deletion of another PAK-like protein kinase involved in cytokinesis, Cla4p. However, the effect was more pronounced in the a1 mating background. New evidence suggests that these mating type dependent defects in *smu1* and *cla4* deletion mutants extend to rates of growth as well, with *cla4* a1 mutant growing slower and *smu1* a2 mutant growing faster when compared to wild type. Also, yeast two hybrid analysis identified two potential Smu1p interacters, Uro1p and Hsl7p, both of which exhibit mating type specific effects. Data suggest that while Uro1p is required for viability, when over-expressed, it also reduces the response to pheromone dramatically in the a2 mating background. Disruption of *hsl7*, causes cell elongation and reduction in the rate of growth, independent of mating background, yet only the deletion in the a1 background shows sensitivity to cell wall inhibitors. Thus, mating type dependent effects provide an interesting line of investigation into the overall control of mating and pathogenicity in *U. maydis*.

182. Exploring interactions among ammonium transporters of fungi.

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Fungal proteins that sense carbon and nitrogen availability interact with conserved signaling pathways to regulate mating and the transition from budding to filamentous growth. Ammonium transporters (e.g., Mep1,2,3 from *Saccharomyces cerevisiae* and Ump1 and Ump2 from the corn smut, *Ustilago maydis*) are important for uptake of ammonium as a nitrogen source. Moreover, Mep2 and Ump2 can sense low ammonium availability and transmit this signal to trigger the dimorphic switch. Interestingly, we have found that *U. maydis* cells over-expressing the *ump2* gene grow filamentously in carbon- and nitrogen-replete conditions. Similar findings in *Candida albicans* and *S. cerevisiae* suggest that ammonium limitation per se might not be required for induction of the dimorphic switch in response to ammonium. We used split ubiquitin to further explore possible interactions among Ump1, Ump2, and their putative signaling pathway(s). The results suggest that *U. maydis* transporters Ump1 and Ump2 physically interact with themselves and with each other. Moreover, they appear to have weak interaction with a Rho1 homologue, thus suggesting an avenue for the signaling cascade. We are also investigaing the ammonium transporter homologues in another smut fungus, *Microbotryum violaceum*. One of the genes, *mepA*, complemented the transport function in the *S. cerevisiae mep1,2,3* triple deletion mutant; however, it was not able to complement the filamentation defect on low ammonium for either this mutant or the *ump2* mutant of *U. maydis*. Predicted amino acid sequence of a second homologue, MepC, reveals various levels of similarity to other well studied ammonium transporters and is currently under investigation to evaluate its possible roles in transport and filamentation.

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183. Physiological inducer and *cis*-elements required for the cellobiohydrolase I gene induction via XlnR-independent signaling pathway in *Aspergillus aculeatus*.

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We are studying on the regulation mechanisms of cellulase and hemicellulase gene expressions in *Aspergillus aculeatus*, which secretes enzymes that synergistically hydrolyze pulp with *Trichoderma* cellulases. We found that the inductions of cellobiohydrolase I (*cbhI*) and carboxymethylcellulase 2 (*cmc2*) genes were regulated by namely XlnR-independent signaling pathway in *A. aculeatus*, while inductions of all cellulase and hemicellulase genes were under control of the common regulatory system involving Xyr1, XlnR homolog in *T. reesei*. To figure out this novel signaling pathway, we investigated regarding a physiological inducer and *cis*- elements required to induce the *cbhI* expression. Quantitative analyses of *cbhI* transcripts revealed that cellobiose induced it most effectively among sophorose, laminaribiose, cellobiose, gentiobiose, lactose, and galactose. Using GUS reporter fusions, analyses of truncated and mutated *cbhI* promoters revealed that three regions were necessary for effective cellulose-induced transcription of the *cbhI*, all of which contained the conserved sequences within CeRE identified as the upstream activating element essential to inductive expression for the *eglA* in *A. nidulans*[1]. Possible sequence conserved in these regions is 5 f-CCGN$_2$CCN$_6$GGA-3 f. 1. Endo, Y. et. al. *Biosci. Biothechnol. Biochem*., **72,** 312-320, 2008

184. Oxygen Sensing in *Cryptococcus neoformans*: How Many Pathways are there?

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Cryptococcus neoformans is an opportunistic fungal pathogen, which requires atmospheric levels of oxygen for optimal growth. *C. neoformans* enters the host via respiratory route and disseminates to central nervous system causing meningoencephalitis mostly in immuno-compromised patients. In order to investigate the oxygen sensing mechanisms in *C. neoformans*, we took two different approaches; 1) screening for the T-DNA insertional mutants directly under low oxygen conditions and 2) screening for hypoxia-mimetic cobalt chloride ($CoCl_2$) sensitive mutants. In *C. neoformans*, we have shown previously, under low sterol or low oxygen conditions, Sre1 and Scp1 proteins (homologs of the mammalian SREBP (sterol regulatory element-binding protein) and SCAP (SREBP cleavage-activating protein) respectively) regulate many of the genes involved in ergosterol biosynthesis and iron homeostasis. By direct screening of the mutant library in low oxygen conditions at 37oC, we isolated additional mutants that showed defects in SREBP processing. Many of the genes affected in these mutants have shown to be involved in the SREBP processing in mammalian systems while some genes have shown to be involved in SREBP processing only in *C. neoformans* so far. Interestingly, all the mutants obtained through $CoCl_2$ sensitivity screening had a growth defect under low oxygen conditions at 37^{o}C. The majority of mutants are compromised in their mitochondrial function. Our findings indicate cobalt chloride sensitivity and/or sensitivity to low oxygen conditions are linked to mitochondrial function, sterol and iron homeostasis, ubiquitination and the ability of cells to respond to ROS. These findings imply that multiple pathways are involved in oxygen sensing in *C. neoformans*.

185. Distinct and overlapping transcriptional responses to calcium and alkaline exposure in *Aspergillus fumigatus*

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Calcium (Ca^{2+}) signalling and pH homeostasis contribute to pathogenesis, in a range of fungi, via conserved regulatory molecules. A deeper understanding of the sensing events upstream of these cellular stimuli will inform exploitative interventions from a therapeutic perspective. However, such studies have not yet been reported for the pathogenic mould *Aspergillus fumigatus*. We performed multiple phenotypic screens to determine the physiological response of *A. fumigatus* to pH stress in both the presence and absence of added Ca^{2+}. Depletion of calcium strongly inhibits growth at all pHs such that Ca^{2+} is indispensable for *A. fumigatus* growth under both acidic and alkaline conditions. In addition, a microarray study was performed where *A. fumigatus* mycelia were shifted to either 200 mM $CaCl_2$ or pH 8.0 over a time course of 60 minutes. The datasets were analyzed independently and comparatively in order to obtain stress-specific responses and examine correlation between the two mechanisms, respectively. While calcium adaptation occurs within 15 minutes of challenge, pH adaptation requires one hour or longer. A comparison of the responses revealed clusters of genes whose transcription is up- or down- regulated in both cases.

186. A homeoprotein, NrsA represses sexual development of *Aspergillus nidulans*

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The *nrsA* gene is predicted to encode a novel 293 aa protein with a homeobox domain at the C terminus. Deletion of *nrsA* resulted in the increase of cleistothecial development even in the presence of stresses and in the reduction of conidia formation. Conversely, forced expression of *nrsA* blocked formation of cleistothecia completely. Over expression of *nrsA* in *nsdD* multicopy strain exhibited the phenotype similar to that of the wild type. And double over-expression of *nsdD* and *nrsA* inhibited both sexual and asexual development. These results suggest that NrsA act as a negative regulator of sexual development and may function as a member of potent antagonists of NsdD. The *nrsA* mRNA was hardly detectable during vegetative growth. It accumulated during asexual sporulation but was very unstable. More than ten GATA sequences were found in the promoter region of*nrsA*. *In vivo* ChIP assay revealed that the NsdD bound to the promoter of *nrsA*. The expression of *nrsA*increased in *nsdD* deletion mutant or *nsdD*/*veA* double deletion mutant, indicating that NsdD represses the transcription of the *nrsA* gene. Taken together, we propose that the homeo-domain protein, NrsA administrates coordinated regulation of two distinct developmental programs in *A. nidulans* and is under the antagonistic control of NsdD and VeA.

187. A carbonic anhydrase CanB is necessary for CO_2 sensing in *Aspergillus nidulans*.

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In *Aspergillus nidulans*, hypoxic condition is one of the most important environmental factors for generating fruit bodies or cleistothecia. To study relationship between fungal development and hypoxic stress condition as well as other gaseous status, DNA microarray experiment was performed using *A. nidulans* 70-mer oligo microarray chip. As a result, we identified that a gene encoding carbonic anhydrase (CA), which mediate reversible interconvertion between gaseous CO_2 and bicarbonate ions (HCO_3-) for maintaining cellular homeostasis, is up-regulated at hypoxic condition. CA is ubiquitous and grouped into four general classes that are alpha, beta, gamma, and delta-CA. Genome sequence of *A. nidulans* allowed us to identify three conserved beta-CAs designated as *canA* (CA in *A. nidulans*), *canB* and *canC*, respectively. Interestingly, *canC* may not be functional because a fungal transposon was integrated into the 3¡¯ end of *canC* locus. Knock-out of the *canB* gene resulted in leathal phenotype under the normal air condition (~0.036% of CO_2). However, this phenotype was recovered when the mutant was cultured under high CO2 (~5%) condition, suggesting that the *canB* gene is required for CO_2 sensing in *A. nidulans*. This work was supported by grant from KOSEF (R1-2006- 000-11204-0).

188. Differential gene expression during sclerotium formation and development in the basidiomycete phytopathogen *Sclerotium rolfsii*.

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Sclerotium rolfsii, the causal agent of southern blight or white mold, is a basidiomycete plant pathogen with a host range of over 400 species. *S. rolfsii* is a soilborne pathogen that survives and is spread from season to season as small resistant sclerotia. Although much is known about the environmental conditions required to stimulate sclerotium production, little is known about the molecular mechanisms that are crucial to this process. To this end, we created an EST library of sequences differentially expressed during sclerotium formation and development using Suppression Subtraction Hybridization PCR (SSHP). Roughly 200 of the 2,000 library clones were identified as containing sequences up- or down-regulated during sclerotium development. Of these sequences, we identified 51 clones that correspond to 20 fungal protein-encoding genes present in the National Center for Biotechnology Information (NCBI) database, and 44 clones that have no similarity to any proteins or genes present in the NCBI databases. Sclerotium upregulated sequences include phosphatidylserine decarboxylase and lectin genes, which may be involved in aminophospholipid biosynthesis and signaling, respectively. Downregulated sequences include genes that encode two putative signaling proteins and a cytochrome c oxidase gene. Confirmation of differential expression of these genes is being carried out by northern and real-time PCR analyses. The ultimate goal of our research is to identify genes and pathways that trigger sclerotium formation and may provide new targets to disrupt the life cycle of *S. rolfsii*, stopping or slowing the season-to-season spread of this pernicious pathogen.

189. Engineering intracellular metabolism by altering gene expression of *Aspergillus oryzae*

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Aspergillus oryzae is one of the most important organisms in Japanese fermented food industry. Though it hardly produces secondary metabolites, related organisms are producers of diverse metabolites. A major objective of our project is to develop a system using *A. oryzae* to generate diverse metabolites. Combinatorial biochemistry approach by introducing exo-genous genes and fermentation techniques are combined. To reveal regulation of metabolic pathways in *A. oryzae* under various conditions, gene expression profiles under condition of submerged culture, solid media and so on were analyzed by DNA micro array. A series of deletion mutants of transcription factor have been obtained by systematic gene targeting. More than 200 mutants were tested for their ability to produce some metabolites. Several mutants showed lack of production or different dependency on environmental nutrient conditions. The factors affecting expression level of metabolic genes are being studied. To develop the system, novel vectors and host strains of *A. oryzae* have been constructed. We have replaced promoter regions of some metabolic genes and successfully altered the level of some metabolite productions. To introduce genes, the correct detection of splicing position is important. We have analyzed motifs of splicing in *A. oryzae* by comparing EST and genome sequences.

190. B-regulated sexual development and the sugar transporter Sts1 in *Schizophyllum commune*.

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Mushroom development in the fungus *Schizophyllum commune* is normally the result of a sexual interaction between two individuals differing at what are termed the *A* and *B* mating type loci. *sts1* is a putative sugar transporter gene also implicated in the regulation of mushroom development. Null (Delta-) mutant strains lacking functional copies of *sts1* displayed severely attenuated mushroom production. When Delta-*sts1* strains were outcrossed, many of the Delta-*sts1* null progeny displayed a “flat” phenotype, suggestive of an inappropriately-activated B mating type pathway. Activation of this pathway normally requires the mating of two haploid individuals with different B mating type specificities. Specific *B* loci encode both a G-protein coupled receptor (GPCR) and small lipopeptide pheromones that do not normally interact within self. We have investigated a possible link between the B mating type pathway and *sts1* by outcrossing our Delta-*sts1* strain with a compatible strain containing an incomplete *B* mating type locus. Progeny from this cross were analyzed by both genetic and molecular approaches. The results of these analyses were consistent with the hypothesis that an intact *B* mating locus inherited from the Delta-*sts1* parent was necessary in order for the “flat” phenotype to be displayed in a Delta-*sts1* null mutant background. Our working model is that the activation of the B pathway by Delta-*sts1* is the consequence of permitting activation of the GPCR by one of its' “self” pheromones. Reconstruction experiments involving the sequential addition of the GPCR and its' cognate pheromones to a *B* null, Delta-*sts1* null strain will be performed to test this model.

191. Identification of putative polyadenylation signals in *Aspergillus oryzae* using EST data.

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In the previous study, we showed that premature polyadenylation within the ORF of AT- rich heterologus genes generally occurred in *Aspergillus oryzae*, and it was prevented by codon optimization (*Appl. Environ. Microbiol.*, **74**, 6538-6546 (2008)). This observation suggested that low mRNA levels of heterologus genes were resulted by the degradation process of mRNA lacking translation termination codon, which is called "nonstop mRNA decay" pathway. In eukaryotes, poly(A) addition site was determined by several sequence elements, and those sequence element have been extensively studied in mammals and yeast. Among those, hexanucleotide AAUAAA and its related sequences located in 10-30 nt upstream of poly(A) site are known as most highly conserved sequence element, which is called polyadenylation signal. However, there were no AAUAAA or its related sequence within ORF of heterologus genes occurred premature polyadenylation. This suggested that another sequence could function as polyadenylation signal in *A. oryzae*. In this study, we constructed a dataset of nucleotide sequences around poly(A) site from EST (expressed sequence tag) data to elucidate the sequence elements that can function as 3'-end processing signals in *A. oryzae*. Furthermore, we examined the 3'-UTR length and nucleotide profile around the poly(A) site by using this dataset.

192. Transcriptome analysis revealing AtfA transcription factor functioning downstream of HogA MAPK cascade in *Aspergillus nidulans*.

Daisuke Hagiwara[1], Yoshihiro Asano[2], Junichiro Marui[1], Akira Yoshimi[1], Takeshi Mizuno[2], and Keietsu Abe[1]. [1] NICHe, Tohoku University, Sendai, Japan, [2] Department of Bioagricultural Science, Nagoya University, Nagoya, Japan.

Fungicides such as fludioxonil and iprodione are specifically applied to filamentous fungi. Group III histidine kinase (HK) is suggested to be a target of these fungicides. Indeed, NikA, an *Aspergillus nidulans* group III HK, deletion mutant exhibited a tolerance to such fungicides. Also, the double deletion mutant of SskA and SrrA, the response regulators (RRs) that function downstream of HKs, was fully tolerant to the fungicides. From these findings, we assumed that the growth inhibitory effects are transmitted through the signaling system NikA-SskA/SrrA. To gain more insight into a physiological role and a signaling mechanism of this system, we conducted a global expression analysis by using DNA microarray. We found that 283 genes are induced (fungicide induced genes, FIGs) and 121 genes are down-regulated (fungicide down-regulated genes, FDGs) in response to fungicide. A large part of these genes are regulated in an SskA- and HogA MAPK-dependent and SrrA-independent manner. Moreover, we found that the transcription of AtfA that is involved in stress responses in conidia was induced by fungicide treatment in an SskA- and HogA-depndent manner. To examine the possibility that AtfA functions downstream of SskA-HogA signaling pathway, we determined the AtfA- dependent FIGs and FDGs by DNA microarray analysis. As a result, 50% of FIGs and 2% of FDGs are regulated in an AtfA-dependent manner. These results suggested that AtfA functions downstream of HogA MAPK and the SskA-HogA MAPK-AtfA signaling pathway plays a role for the regulation of FIGs. The roles of this signaling pathway in fungicidal effect and osmotic stress response will be discussed.

193. Role of a hexokinase-like protein and a p53-like transcription factor in fungal programmed cell death

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We have identified three genes involved in the response to nutrient depletion in the filamentous fungus, *Aspergillus nidulans*. Two of the genes encode non-catalytic hexokinase-like proteins (HxkC and HxkD) and the third gene product (XprG) belongs to a newly defined class of p53-like transcription factors containing an Ndt80-like DNA- binding domain. Ndt80 is yeast transcriptional activator required for progression through meiosis. Vib-1, a regulator of genes required for programmed cell death in *N. crassa* is a homolog of XprG. Genetic evidence indicates that HxkC and HxkD are negative regulators of XprG. HxkD is a nuclear protein and HxkC is associated with mitochondria. Mitochondrial hexokinases have been shown to block apoptosis in mammals and programmed cell death (PCD) in plants. We have evidence that suggests that HxkC may block XprG-mediated PCD in *A. nidulans*. A *hxkC*⁻ null mutant, but not the *hxkC*⁻*xprG*⁻ double mutant, exhibits markers of PCD without exposure to any PCD- inducing agent. We have recently found that XprG is localised to the nucleus during growth in nutrient-sufficient medium but most appears to be associated with mitochondria during carbon starvation, consistent with a role in triggering PCD in response to nutrient limitation. The numbers of genes encoding Ndt80-like DNA-binding proteins varies in different fungi. *S. cerevisiae* contains only one, *NDT80*. The *A. nidulans* genome contains a second *NDT80*-like gene, AN6105. Disruption of AN6015 showed that AN6015, is required for meiosis, like the *S. cerevisiae NDT80* gene.

194. Integration of farnesol signaling in *Candida albicans*: importance of heterogeneous response in population for the promotion of level fitness in the face of oxidative stress.

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C. albicans is an opportunistic pathogen capable of causing a wide range of diseases, from mild mucosal diseases to life-threatening systemic infections. *C. albicans* frequently encounters high levels of oxygen reactive species (ROS) during interactions with other microorganisms and during infection of human tissues, and needs to reliably respond to these assaults to survive. Farnesol, a quorum sensing molecule produced by *C. albicans*, inhibits hyphal growth and protects cells against heat shock by inhibiting the Ras1/cAMP pathway (1). Previous data indicate that farnesol also protects against ROS stress (2). We questioned how farnesol signal is integrated by cells to enhance survival in response to ROS. We hypothesized that heterogeneity induced by farnesol promotes population level fitness in the face of oxidative stress. Mutant analyses were used to determine the respective contribution of the HOG MAP kinase and Ras1/cAMP pathways to farnesol signaling in response to ROS. The two pathways are involved in stress response and are altered by farnesol (1, 3). Then, we used flow cytometry sorting to analyse the resistance to ROS of subpopulations differentially sensitive to farnesol in yeast and hyphal inducing conditions.

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195. The role of MYB transcription factors in the life cycle of *Phytophthora infestans*

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Asexual spore production in the oomycete *Phytophthora infestans* involves distinct temporal and spatial developmental switches which presumbly are promoted by groups of differentially expressed genes. This study focuses on identifying the transcription factors and cognate promoter binding sites that are responsible for sporulation-specific gene expression. Dissections of the promoters of several sporulation-specific genes have identified putative transcription factor binding sites important in their developmental regulation. For example, analyses of the *pks1* gene showed that the motif CCGTTG determines its transcription at an intermediate stage of sporulation. This motif is significantly enriched in promoters highly active in sporangia and specifically binds a protein complex from nuclear extracts of sporangia. As CCGTTG is known to bind MYB transcription factors in animals and plants, such proteins are being characterized in *P. infestans*. MYB transcription factors are proteins with one, two or three incomplete repeats of a conserved DNA binding domain consisting of about 50 amino acids, and those regulating development typically carry two or three tandem repeats. *P. infestans* possesses multiple proteins with two or more tandem repeats, and many of them are differentially expressed during sporulation based on RT-PCR. Phylogenetic analysis is being carried out to determine how these MYBs relate to counterparts in other eukaryotes. Gene silencing experiments are also being conducted to determine whether MYB proteins regulate the spore cycle.

196. Analysis of the response of *Candida albicans* to novel silver-coumarin complexes.

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The yeast *Candida albicans* is one of many opportunistic fungal pathogens capable of causing serious systemic infections in transplant patients, HIV patients and neonates. A significant increase in resistance to conventional anti-fungal therapies has been reported recently. Metal-Based drugs represent a novel group of anti-fungal agents with potential applications for the control of drug-resistant fungal infections. We have synthesized two potent anti-microbial complexes, namely coumarin-3-carboxylatosilver (I) ([Ag (Cca)]) and 4-Oxy-3-nitrocoumarinbis (phenanthroline) silver (I) ([Ag (hnc)(phen)2]). These agents demonstrate anti-*Candida* MIC_{80} values of 35 and 15 microgram/ml respectively and reduce many of the virulence attributes of *C.albicans* including adherence, dimorphism and enzyme secretion. Analysis of the response of *C. albicans* to these agents revealed the activation of the Cap1p pathway. Our results demonstrated nuclear translocation of the Cap1p and the concomitant activation of genes responsible for the oxidative stress response such as *glutathione reductase* (GLR1), *mitochondrial processing protease* (MAS1) and *manganese-superoxide dismutase* (SOD2). The enzymatic activity of both Glutathione reductase and superoxide dismutase has also been analyzed. We also identified the activation of the Hog1p pathway with the activation of genes for *catalase* (CAT1) and *alkyl hydroperoxide reductase* (AHP1). Our results demonstrate for the first time that these silver-coumarin complexes induce oxidative and osmotic stress in *C. albicans* and thus have a mode of action distinct to that of the conventional antifungal agents.

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197. Genome Adaptations for Trichothecene Synthesis in *Fusarium graminearum* Are Revealed by Transcription Factors *Tri6* and *Tri10*.

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Trichothecenes are isoprenoid mycotoxins in wheat infected with the filamentous fungus *Fusarium graminearum*. Some fungal genes for trichothecene biosynthesis (Tri genes) are known to be under control of transcription factors encoded by *Tri6* and *Tri10*. *Tri6* and *Tri10* deletion mutants were constructed in order to discover additional genes regulated by these factors *in planta*. Both mutants were greatly reduced in pathogenicity and toxin production and their phenotypes were restored by genetic complementation with the wild-type gene. Transcript levels for over 200 genes were altered more than two-fold for *tri6* or *tri10* mutants including nearly all known Tri genes. Also reduced were transcript levels for enzymes in the isoprenoid biosynthetic pathway leading to farnesyl pyrophosphate, the immediate molecular precursor of trichothecenes. DNA sequences 5' to isoprenoid biosynthetic genes were enriched for the Tri6p DNA binding motif, YNAGGCC, in *F. graminearum* but not in related *Fusarium* species that do not produce trichothecenes. Differentially regulated genes will be targeted for functional analysis to discover additional factors involved in toxin biosynthesis, toxin resistance and pathogenesis. This project is supported by the National Research Initiative Microbial Functional Genomics Program of the USDA Cooperative State Research, Education and Extension Service.

198. Identification of *Trichophyton rubrum* expressed genes involved in pH modulation.

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Trichophyton rubrum is a dermatophyte fungus that infects human skin, hair and nails, utilizing keratin as nutrient source, secreting a variety of enzymes, such as keratinases, phosphatases, elastases, among others, which may be regulated by the extracellular pH. During infection, this dermatophyte must have metabolic machinery that allows environmental pH modulation, since it has to sense and adapt to the acidic pH of the skin, and to change environment pH to alkaline, enabling the maintenance of the infection. The aim of this study is the identification of *T. rubrum* differentially expressed genes in acid (pH 5.0) and alkaline (pH 8.0) pH. For this purpose, forward and reverse suppression subtractive hybridization (SSH) libraries were constructed, after cultivation of a *T. rubrum* strain on minimal media pH 5.0 or pH 8.0. After differential screening assays, the differentially expressed genes from both libraries were sequenced, annotated, and some genes were validated by Northern blot analyses. Taken together, these results contribute to the understanding of *T. rubrum* extracellular pH response, and the regulatory mechanisms possibly involved in pathogenhost interaction.

199. Repeat induced point mutation and DNA methylation in *Fusarium graminearum*.

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Repeat-Induced Point mutation (RIP) effectively silences repetitive DNA by mutation and subsequent cytosine methylation in *Neurospora crassa*. After fertilization, but prior to karyogamy, the RIP machinery recognizes linked and unlinked duplications and peppers both copies with C:G to T:A mutations. This presumably has severe consequences for the evolution of RIP-competent ascomycetes, as RIP also acts on gene duplications that may serve as "raw material" for gene innovation. The molecular mechanism of RIP is poorly understood; however, Neurospora mutants lacking a DNA methyltransferase homologue, RID, are defective in RIP in homozygous crosses. We are exploring the homothallic *Fusarium graminearum* (teleomorph: *Gibberella zeae*) to isolate recessive RIP mutations. First, we confirmed that *F. graminearum* is as efficient as *N. crassa* in mutating unlinked duplicated genes by RIP. We selfed strains with two or three copies of *hph* and found that 61% and 32% of the isolates, respectively, lost hygromycin resistance. The *hph* genes from 17 Hyg-resistant or Hyg-sensitive isolates were sequenced: 152 of the 154 mutations were C:G to T:A transitions, indicative of RIP. By Southern analyses we found that even heavily mutated *hph* alleles lack methylated cytosines, different from what has been observed in Neurospora. This suggests that heterochromatin silencing in *F. graminearum* is initiated by a different mechanism than in *N. crassa*. Secondly, we found that the *F. graminearum rid* homologue is essential for RIP. This result sets the stage for the isolation of novel RIP mutants by insertional and transposon mutagenesis in *F. graminearum*.

200. Characterization of interactions between and among components of the meiotic silencing by unpaired dna machinery in *Neurospora crassa* using bimolecular fluorescence complementation.

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Bimolecular fluorescence complementation (BiFC) is based on the complementation between two nonfluorescent fragments of the yellow fluorescent protein (YFP) when they are united by interactions between proteins covalently linked to them. We have successfully applied BiFC in *Neurospora crassa* using two genes involved in Meiotic Silencing by Unpaired DNA (MSUD, which acts to silence unpaired chromosomal sequences via an RNA interference pathway) and observed macromolecular complex formation involving only SAD-1 proteins, only SAD-2 proteins, and mixtures of SAD-1 and SAD-2 proteins.

201. Photoreceptor genes in *Phycomyces*

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The zygomycete *Phycomyces blakesleeanus* responses to light include phototropism of the fruiting body, induction of beta-carotene biosynthesis, and regulation of fruit body development. All of the responses to light require the products of the *madA* and *madB* genes. *madA* encodes a protein similar to the *Neurospora* blue-light photoreceptor, zinc-finger protein WC-1, and *madB* encodes a protein similar to the *Neurospora* zinc-finger protein WC-2. MADA and MADB interact to form a complex in yeast two-hybrid assays and after co-expression in *E. coli*, suggesting that the responses to light are mediated by a photoresponsive transcription factor complex. The *Phycomyces* genome contains three *wc 1* genes and four *wc 2* genes, including *madA* and *madB*. In addition, the genome contains a gene, *cryA*, similar to the blue-light photoreceptor cryptochrome DASH. Most of the photoreceptor genes are induced by light, except *madA* and *madB*. *wcoA, wctB*, and *wctD* are induced by low- intensity light, while *wcoB* and *cryA* need high-intensity light for photoinduction. Gene photoinduction is impaired in *madA* and *madB* mutants. The presence of multiple *wc* genes may allow perception across a broad range of light intensities, and formation of specialized photoreceptors for particular photoresponses.

202. Regulation of mushroom formation in the basidiomycete *Schizophyllum commune*

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The basidiomycete *Schizophyllum commune* is used as a model system to study mushroom formation. Genome-wide expression analysis resulted in the identification of several transcription factors which are differentially regulated during mushroom development. A deletion mutant of one of these transcription factors (*Reg7*) showed abnormal regulation of mushroom formation: fruiting bodies developed earlier and in greater numbers. This clearly shows that *Reg7* is involved in regulation of mushroom formation. Genome-wide expression analysis also resulted in the identification of several structural genes which are specifically expressed in one or more stages of mushroom development and therefore serve as developmental markers. Among these genes are *lccA* (laccase), *SC7* (coding for a hydrophilic cell wall protein), *agglutinin* (lectin) and *SC1* and *SC4* (both hydrophobins). We have cloned the promoters of these genes in front of the red fluorescent reporter gene *dTomato* to study their spatial expression during fruiting body development. Spatial localisation of fluorescence was monitored by (time-lapse) fluorescence microscopy. Promoter analysis of these mushroom-specific genes resulted in the identification of several putative transcription factor binding sites, which are currently being studied.

203. Regulation by light of conidiation genes in *Aspergillus nidulans*

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The ascomycete fungus *Aspergillus nidulans* is a model organism for the research on conidiation. Red and blue light stimulate conidiation in *A. nidulans,* but mutations in the veA gene allow conidiation in the dark. Conidiation in *A. nidulans* is controlled by a master regulator, the product of the brlA gene. Many gene products act upstream of brlA, presumably allowing the synthesis of chemicals or allowing the transduction of environmental signals, to trigger brlA transcription and conidiation. The regulation of conidation by light may occur through activation of the expression of some regulatory genes. We have shown that the expression of several conidiation genes, including brlA, fluG flbA, flbB and flbC, is activated by light. The level of photoinduction was moderate, between 4-10 fold, and changed with the age of the culture and the genetic background. Some genes, like brlA, are activated by light regardless of age, but flbA was only induced by light in 18 h-mycelia, while flbC was only induced in 25 h-mycelia. Mutation in veA did not prevent photoactivation of gene expression, and an increase in light-dependent mRNA accumulation was observed for flbA and flbC. The veA mutant did show an increase in the accumulation of some conidiation mRNAs in the dark. The activation by light of conidiation genes would provide increased amounts of regulatory proteins for conidiation clarifying the role of light on conidation.

204. *Cryptococcus neoformans* Rim101 homolog is involved in capsule and iron regulation.

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Pathogenic microorganisms such as *Cryptococcus neoformans* must be able to adapt to hostile host conditions including iron starvation. The host uses iron sequestration as part of the innate immune system, and iron deprivation is a powerful host-specific signal that results in the induction of microbial virulence-associated phenotypes such as capsule. Understanding the interplay of host iron availability and microbial iron acquisition has recently been recognized as an essential component of microbial pathogenesis. When *C. neoformans* enters a host, it responds to specific host signals and activates capsule production using the cAMP/PKA pathway. This capsule is required for evading host defenses and surviving in the host. In this study, we have identified a specific *C. neoformans* transcription factor, Rim101, that regulates both iron homeostasis and capsule production in response to PKA stimulus. The data support the hypothesis that PKA-activated transcription factors offer a previously unknown mechanism for interpreting host-specific signals through the cAMP/PKA pathway. These transcription factors are essential in responding appropriately to host- specific signals and regulating capsule production and iron homeostasis.

205. Genome-wide mapping of direct targets for the circadian regulator WHITE COLLAR- 2.

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Circadian oscillators control rhythmic expression of numerous target genes and coordinate rhythms with daily oscillations in the environment, e.g. light-dark cycles. *Neurospora crassa* expresses two PAS-domain/GATA-type Zinc finger proteins, WHITE COLLAR-1 (WC-1) and WHITE COLLAR-2 (WC-2). WC-1 is both a blue light photoreceptor and transcription factor. It directly affects transcription of clock-controlled genes (*ccg*) when bound to WC-2 in the "White Collar Complex" (WCC), a major component of the FRQ/WCC oscillator. Regulatory mechanisms responsible for the regulation of hundreds of *ccg*'s by oscillators remain elusive. One model for how circadian clocks regulate downstream *ccg*'s proposes the existence of genes that are direct targets of WCC and whose products lie within circadian output pathways. We took a direct biochemical approach to define immediate targets for WCC by mapping the genome-wide distribution of WC-2 after light induction. Chromatin immunoprecipitation followed by high throughput DNA sequencing (ChIP-Seq) of WC-2-bound DNA identified all previously known target sites for WC-2 (near *frq*, *vvd* and *al-3*). From among the >400 regions with WC-2 enrichment, we selected ~60 target sites for further studies. WC-2 binding sites mapped predominantly upstream of genes whose expression was inducible by light. Genes encoding transcription factors were over- represented when compared to previous transcriptional studies. When deleted, none of the target genes studied resulted in defects in circadian rhythms, suggesting that they do not affect the clock but instead are regulators within output pathways. Our work opens the door to mechanistic studies that will integrate transcriptional regulation, chromatin structure and cell cycle control with the normal function of the circadian clock.

206. A crossover hotspot near *cog* is a preferential recombination termination site

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There seems to be a crossover hotspot near to, but distinct from, the recombination hotspot *cog*, as an excess of crossovers was found within a 200 bp region near the 3' end of *his-3* during analysis of 150 unselected octads. Homozygous deletion of this region reduces crossing over between *his-3* and *ad-3* to about 85% of the normal level, while increasing the adjacent *his-3* to *lys-4* interval to 125%. In addition, the deletion appears to increase gene conversion in *his-3*. We conclude that recombination events initiated at *cog* are more likely to terminate within the 200 bp region, and some of these terminations will be resolved as crossovers, resulting in a cluster of crossovers at this location. Removal of the termination site increases the chance that an event will reach *his- 3*, increasing the frequency of conversion, or extend past *his-3* to yield a crossover between *his-3* and *lys-4*. We wonder whether crossover hotspots detected in other organisms may also reflect preferential termination sites, rather than directly indicating local initiation of recombination. This work was supported by a grant from the Australian Research Council.

207. DopA regulates mycelial growth and conidial development in *Aspergillus nidulans* and *A. fumigatus*

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Aspergillus fumigatus is a predominant airborne fungal pathogen with worldwide distribution. It is the causal agent of invasive aspergillosis, a life threatening disease in immuno-compromised patients. The main objective of this study is to investigate processes controlling the establishment and maintenance of invasive polarized hyphal growth in the opportunistic human pathogen *A. fumigatus* . This information will elucidate mechanisms by which filamentous growth may function as a virulence factor. *Aspergillus nidulans* is closely related to *A. fumigatus* and we use this model fungus for comparative studies. A *dopA* null mutation in *A. nidulans* clearly indicates that DopA affects hyphal polarity, conidiation and ascospore formation. Polar growth of the mycelium is an important virulence factor. We found that a *dopA* null mutation in *A. fumigatus* resulted in strong polar growth defects and reduced conidial production. As conidia are essential air-borne propagules and invasive growth is a key factor in pathogenesis, *dopA* appears to be an important pathogenicity factor for the *A. fumigatus* . In this study, we are currently studying the functional role of *A. fumigatus* DopA protein in controlling cell polarity and pathogenicity of this fungus.

208. Effects of stress stimuli on "transposability" and post-transcriptional modifications of mRNAs from DNA transposon *Crawler* in *Aspergillus oryzae*.

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An active DNA transposon *Crawler* isolated from the genome of industrially important fungus *Aspergillus oryzae* transposes under extreme stress conditions. The mRNA analysis of *Crawler* in the conidia revealed that cryptic splicing and premature polyadenylation of the mRNA occurred in the normal culture condition. In the present study, we analyzed the relationship between transposition events and proportional change in post-transcriptional modifications of the *Crawler* mRNA by RT-qPCR under various stress stimuli. These results suggested that post- transcriptional regulation for *Crawler* tends to be inhibited by stress treatment of $CuSO_4$ and heat shock, which stimulated the transposition events in conidia allowing the full-length and active transposase to be produced. Even extreme stress such as UV, H_2O_2 or metal ions except Cu^{2+} could not lead to the transposition of *Crawler* resulting from insufficient changes for mature mRNA molecules. Moreover the effects of stress stimuli inducing the transposition events of *Crawler* on the splicing for several genes responsible for metabolism, cellular structure or RNA processing were also examined to understand the control mechanism against the active transposon.

209. Aflatoxin biosynthesis of *Aspergillus parasiticus* under calcium stress requires the expression of *crzA* which encodes a putative calcineurin response regulator.

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The control of calcineurin-dependent gene expression is via a transcription factor, CrzA. Two morphologically different *Aspergillus parasiticus* strains, one producing aflatoxins, abundant conidia but few sclerotia (BN9) and the other producing *O*- methylsterimatocystin (OMST), copious sclerotia but a low number of conidia (RH), were used to assess the role of *crzA* by gene disruption. Increased concentrations of lithium, sodium, and potassium impaired conidiation and sclerotial formation of the RH?crzA mutants, but they did not affect conidiation of the BN9?crzA mutants. Vegetative growth and asexual development of both ?crzA mutants were hypersensitive to increased calcium concentrations. Calcium supplementation (10 mM) resulted in 3-fold and 2-fold decreases in the relative expression of the endoplasmic reticulum calcium ATPase 2 gene in the BN9 and RH parental strains, respectively, but changes in both ?crzA mutants were less significant. Compared to the parental strains, the ?crzA mutants barely produced aflatoxins or OMST after the calcium supplementation. The relative expression levels of aflatoxin biosynthesis genes, *nor1*, *ver1*, and *omtA*, in both ?crzA mutants were decreased significantly, but the decreases in the parental strains were at much lower extents. CrzA is required for growth and development and for aflatoxin biosynthesis under calcium stress conditions.

210. *Cryptococcus neoformans* Cdk-related kinase Crk1 plays a negative role in mating differentiation process

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Cryptococcus neoformans is a heterothallic basidiomycete and grows vegetatively as yeast. Under nitrogen limitation conditions, strains of opposite mating types, *MAT*a and *MAT*£\, produce conjugation tubes and fuse to form sexual dikaryotic filaments with clamp connections. Cwc1 and Cwc2 are two central photoregulators of *C. neoformans* that form complex to inhibit the formation of sexual filaments in response to light. *Agrobacterium*-mediated insertional mutagenesis screen was used to dissect the light regulatory networks and search the components interacting with or functioning downstream of the Cwc1/Cwc2 complex in the pathway. We characterized mutant DJ22, which suppressed the light dependent *CWC1* overexpression phenotype, and T-DNA was found to integrate at the *C. neoformans CRK1* gene, a homologue of *Saccharomyces cerevisiae IME2* and *Ustilago maydis crk1*. Ime2 is a meiosis-specific gene with the conserved Ser/Thr kinase domain and the significant TXY dual phosphorylation site. Consistent with the findings of other suppressors in our screen, *C. neoformans* Crk1 played negative roles in the mating process. Mating efficiency was increased in the *crk1* mutants and dikaryotic filaments, basidia, and basidiospores were also produced earlier in the crosses involved the *crk1* mutants. Elevation of the *CRK1* mRNA level conversely inhibited sexual differentiation. On the other hand, monokaryotic fruiting was defective both in the *MAT*£*crk1* mutant and *CRK1* overexpression strains. Interestingly, the production of capsule and melanin was also defective in the *MAT*a and*MAT*£\ *crk1* mutants. Our studies demonstrate that mating process is negatively regulated by the *CRK1* gene in *C. neoformans*, and *CRK1* may also play additional roles in other physiological process.

211. Cellophane agar culture for real-time PCR analysis of gene expression early in the sexual development of *Schizophyllum commune*

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Multiple developmental events occur during the initial stages of sexual development in Schizophyllum commune. The processes of septal dissolution, nuclear migration, nuclear pairing and hook cell mediated cell division are established as strains of compatible mating type begin to interact and culminate with the formation of of a dikaryon. Analysis of gene expression changes during these initial stages has been hampered by the traditional mating technique of transferring small inocula into close proximity on semisolid growth media. The progress of development is difficult to monitor under these conditions and even if the mating is established on cellophane agar, the amount of mycelia for genetic analysis is limited. We have developed a cellophane agar based mating protocol where colonies of compatible mates are established at a distance on a common cellophane agar culture and allowed to grow toward one another over time. In this system, the cultures can be monitored microscopically for developmental changes, the exact moment of hyphal interaction between the two mates can be easily predicted, and sufficient mycelia is available for real-time PCR Analysis of gene expression. Using this technique we have characterized dynamic changes in the expression of ddPCR04_05 10b, a gene with homology to the cytochrome P450 family, over the time period from minus 48 hours pre hyphal-contact to 72 hours post contact. Interestingly, the mRNA levels of this gene are depressed pre-hyphal contact and recover to near pre- mating levels prior to establishment of the dikaryon.

212. Convergent evolution of morphogenetic processes in fungi

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Recently, we showed that the same molecular machinery containing a tetraspanin and a NADPH oxidase has been recruited in two different fungal species for the same purpose: exiting from a melanized re-enforced cell at a focal weakened point. Pls1 tetraspanins were shown for some pathogenic fungi to be essential for appressorium-mediated penetration into their host plants. *Podospora anserina*, a saprobic fungus lacking appressorium, contains a Pls1 gene. Inactivation of *PaPls1* demonstrates that it is specifically required for the germination of ascospores in *P. anserina*. These ascospores are heavily melanized cells that germinate through a specific pore. On the contrary, *MgPLS1* has no role in the germination of *Magnaporthe grisea* nonmelanized ascospores but is required for the formation of the penetration peg at the pore of its melanized appressorium. *P. anserina* mutants with mutation of *PaNox2*, which encodes the NADPH oxidase, display the same ascospore-specific germination defect as the *deltaPaPls1* mutant. The analysis of the distribution of *PLS1* and *NOX2* genes in fungal genomes shows that they are either both present or both absent. Altogether, these results indicate that Pls1 and Nox2 are two players of a conserved molecular machinery which is required for the emergence of polarized hyphae from melanized reinforced structures. In addition, we also demonstrated that beside the evolutionary conserved function described above, the PLS1/NOX2 machinery is also likely involved in specialized cellular functions such as nutrient acquisition by *P. anserina*.

213. Investigation of post-translational processing during nitrogen metabolism in *A. nidulans*

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From transcriptomics and proteomic studies into nitrogen metabolism and its regulation in *A. nidulans* we identified a wide variety of genes and proteins showing differential expression. Several of these may function in post-translational regulation: two 14:3:3 proteins, two F-box proteins and a putative Hsp90 co-chaperone. The respective genes were deleted and the phenotype assessed. Northern analysis revealed that mutations in all five genes affect the rate of nitrogen metabolite repression and derepression, when monitoring areA and niaD transcript levels. Western analysis revealed that disruption of the hsp90 co-chaperone and 14:3:3 proteins significantly affect AreA protein. In the wild type different forms of AreA are observed on SDS PAGE, and the proportion varies with nitrogen regime. In these mutant strains the high molecular weight form of AreA is significantly diminished under all growth conditions, suggesting it has a major role in an uncharacterised post-translational event. To investigate the intracellular localisation of the key transcription factors strains expressing GFP-tagged AreA and MeaB have been constructed. Initial experiments show that AreA is localised to the nuclei specifically under conditions of nitrogen starvation in wild type. This pattern of localisation is aberrant in some of the mutant strains, which supports our hypothesis that post-translational events are important in modulating AreA function in vivo.

214. Analysis of *nrdA*, a negative regulator of differentiation in *Aspergillus nidulans*.

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Differentiation into specific cell types and organs requires genetic events turned on or off by external signals as well as internal cues. Continuous activation of growth signals as seen in dominant activating *fadA* G-alpha and its regulator *flbA* mutants inhibited differentiation and caused autolysis in *A. nidulans*. We found that the C_2H_2 zinc-finger transcription factor *nrdA* (a negative regulator of differentiation; formerly msnA) acted downstream to *fadA*. Introducing the *nrdA* null mutation in ¥Ä*flbA* mutants rescued the *flbA* mutant phenotype, producing conidia and protecting from autolysis of ¥Ä*flbA*. *nrdA* exhibited reduced colonial growth, enhanced asexual and sexual sporulations, whereas over-expression of NrdA caused a fluffy-autolytic phenotype. NrdA repressed the expression of *brlA*, a key transcription factor for asexual differentiation by direct binding to the *brlA* promoter. Our data imply that NrdA is required for growth and represses differentiation during growth.

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215. Mating type genes control self/non-self recognition during fertilization, karyogamy and meiosis in the filamentous fungi.
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Aspergillus nidulans matA is a homolog of the human sex-determining factor SRY. The developmentally regulated *matA* gene shows strict chromosomal position effect, suggesting that epigenetic events could be involved in silencing its expression in a sub-population of cells. This chromosomal domain includes other coordinately regulated genes, one of which is a non-coding RNA, jgaA. This non- coding RNA is a member of a large family of eukaryotic RNAs that represent genomic "dark matter" and which have potential roles in gene silencing. Transcription of jgaA and SYG1, a human homolog of the Xenotropic and polytropic retrovirus receptor (XPR1), are convergent with a 30 bp overlap. An over-expression strain and a gene deletion strain of the non-coding RNA have been used to investigate the biological functions of this non-coding RNA. Northern Blot analysis demonstrated that jgaA is co-regulated with matA during sexual development in A. nidulans. A gene deletion of the jgaA shows a 42% decrease in the number of cleistothecia produced per plate. Quantitative RT-PCR has revealed an up-regulation of the G protein-couple receptor (gprB), and change the expression sequence of SYG1. High expression levels of SYG1 were maintained throughout development in the deletion strain. In conclusion, jgaA could be important for sexual development in Aspergillus by an unknown molecular mechanism.

216. AtfA a component of the SakA MAPK pathway regulates oxidative stress response, development and spore viability in *Aspergillus nidulans*.
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Fungi utilize a phosphorelay system coupled to a MAP kinase module as a major mechanism for sensing and processing environmental signals. In *Aspergillus nidulans*, the response regulator SskA transmits osmotic and oxidative stress signals to the MAPK SakA. Here we characterize the *atfA* gene, encoding a transcription factor of the ATF/CREB family, and analyze its role as a downstream component of the SakA pathway. Although AtfA is localized in the nucleus even in the absence of SakA, mutants lacking SakA (*Delta-sakA*) or AtfA (*Delta-atfA*) show similar patterns of osmotic and oxidative stress sensitivity, derepression of sexual development and decreased asexual spore (conidia) viability. AtfA is required for the expression of several genes, and for the accumulation of the spore-specific catalase CatA and the MAPK SakA in conidia, suggesting that decreased spore viability is related to the lack of SakA.

217. Genome-wide expression profiling analysis of transcription factor genes in *Magnaporthe oryzae*
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Magnaporthe oryzae is filamentous ascomycete that causes rice blast disease. The fungus is an important model organism for investigating fungal pathogenicity owing to its genetic tractability and availability of genomic sequence information. Although much effort has been focused to understand the molecular nature of pathogenicity, little is known about the mechanisms of gene regulation at genome level in this fungus. As a first step, we initially identified all putative transcription factors using bioinformatic tools in the fungal genome and achieved them in the database, Fungal Transcription Factor Database (FTFD, http://ftfd.snu.ac.kr). Here, we present expression profiling of 250 *M. oryzae* transcription factor genes during fungal development, and under biotic and abiotic stress conditions using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). These 32 conditions include cellular developments including conidial germination to infectious growth in the host cells, nutrient uptake and utilization, oxidative stresses, different pHs, ionic stresses, DNA damage, phenolic compounds, catalase inhibitor, cell wall stress, microtubule binding chemical and heat shock stresses. Additionally, we evaluated the expression stability of seven housekeeping genes from *M. oryzae* under the conditions. Our comprehensive analysis of expression profiling of transcription factor genes would provide a new paradigm to decipher molecular mechanisms of gene expression and pathogenicity in the rice blast fungus.

218. Mating types and sexual cycle of *Arnium arizonense*.
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Arnium arizonense has been reported to display an unusual life cycle. According to Mainwaring and Wilson (1968, Trans. B. mycol. Soc., 51, 663-677), the binucleate, penultimate cell of a crozier forms an ascus, but the two nuclei do not fuse; instead they divide and the resulting eight nuclei are enclosed in four large, black and four small, hyaline ascospores. No meiosis occurs, although morphological changes in the chromosomes are reminiscent of those associated with meiosis in normal Pezizomycotina (Mainwaring, 1971, Arch Mikrobiol, 75, 296-303). The fungus is therefore apomictic and the entire life cycle is carried out in the haploid phase. Molecular phylogeny based on the ITS clusters *A. arizonense* with the homothallic *Podospora curvicolla* and *Podospora setosa* and close to the heterothallic *Podospora anserina*. This prompted us to clone the mating type of *A. arizonense* by low stringency hybridization with the idiomorphic sequences of *P. anserina*. A single DNA fragment hybridizes with both probes, indicating that *A. arizonense* contains linked counterparts of the *P. anserina* idiomorphs. The *A. arizonense* mating-type sequence will be presented together with detailed cytological observations of the apomictic life style of our isolate.

219. The Hog-related signal transduction cascade regulates cell wall integrity and melanin biosynthesis in the plant pathogen *Botrytis cinerea*.
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Hog1-like fungal signal transduction cascades are classically involved in adaptation to osmotic and oxydative stress, fungicide resistance, and development. In the plant pathogen *Botrytis cinerea* the class III histidine kinase (HK) Bos1 and the downstream MAP kinase (MAPK) Sak1 are important virulence factors, necessary for plant penetration and necrosis development. We have shown that in this ascomycete, the HK negatively regulates Sak1-phosphorylation. Several phenotypes, such as fungicide resistance, are controlled by the Bos1 HK independently of the Sak1 MAPK. In this work we present evidence that in *B. cinerea* this Hog1-type MAPK is involved in cell wall integrity, instead of the Slt2-type MAPK Bmp3. Both *bos1* and *sak1* mutants produce lower amounts of protoplasts and exhibit modified sensitivities towards cell wall interacting compounds compared to the wild type strain. Immunostained microscopic observations confirm cell-wall modifications. Sak1 is phosphorylated upon cell wall challenging, suggesting that Sak1 perceives a cell-wall integrity signal. Phenotypic analyses show that the Bos1-Sak1 cascade regulates melanin biosynthesis. The *bos1* mutant produces much higher amounts of melanin than the wild type, early during the exponential growth phase, whereas the *sak1* mutant is delayed in melanin production compared to the wild type. Expression analysis of melanin biosynthesis genes corroborates the phenotypic results, e.g. while in the wild-type *pks13* expression is limited to one time point at the beginning of stationary phase, it is constitutively expressed in the *bos1* and *sak1* mutants. Mutant strains of the other MAPKs of *B. cinerea* seem to lack melanin production what can be linked to the opposite phenotype observed for the *bos1* mutant. Western-blot analyses show increased amounts of phosphorylated Slt2-like MAPK Bmp3, in both *bos1* and *sak1* mutants. Altogether these results indicate a complex regulatiory scheme of MAPK cascades in this phytopathogenic fungus where the conserved MAPKs do not always play their "traditional" roles.

220. Transcriptional Loops Meet Chromatin: a Dual-Layer Network Controls White- Opaque Switching in *C. albicans*
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Candida albicans is a diploid opportunistic human fungal pathogen causing superficial and systemic infections. The phenotypic plasticity that allows *C. albicans* to adapt to various host niches is considered a major virulence attribute. *C. albicans* can exist as a unicellular yeast, but also able to form pseudo- as well as real hyphae morphologies. Furthermore, *C. albicans* is able to undergo a reversible switch between two distinct cell morphologies called white and opaque, which are considered as different transcriptional states of cells harboring identical genomes. The present model of switching regulation includes the bistable expression of a master switch gene that is controlled by multiple transcriptional feedback loops. The major goal of our studies was to identify novel histone-modifying enzymes implicated in white- opaque switching in *C. albicans* Hence, we constructed homozygous deletion mutants all putative histone-modifying genes in a background homozygous for the *MTL* locus. We then assayed the impact of gene deletions on the white-opaque and on the opaque-white switching frequencies. Here, we show that chromatin-modifying enzymes constitute an additional regulatory layer of switching. We identify chromatin modifiers as novel switching modulators. Epistasis analysis mapped the genes into at least two distinct pathways, some of which overlay the known transcriptional network. The conserved Set3/Hos2 histone deacetylase complex was identified as a key regulator that relies on the methylation status of lysine 4 on histone H3 in switching modulation. We propose that chromatin modifications may integrate environmental or host-derived stimuli through underlying transcriptional circuits to determine cell fate in *C. albicans* This work was supported by a grant from the Christian Doppler Research Society, the transnational ERA-Net Pathogenomics project FunPath (Austrian Science Foundation FWF-I125-B09). DH is a Vienna Biocenter PhD Student Fellow.

221. The *nsdC* gene a encoding a novel C2H2-type zinc finger protein is a key regulator of development in *Aspergillus nidulans*.
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The *nsdC* gene which was predicted to encode a putative transcription factor carrying a novel type of zinc finger DNA binding domain consisting of two C2H2s and a C2HC motif that are highly conserved in most fungi has been characterized. While the deletion of *nsdC* resulted in the complete loss of fruiting body formation, over- expression of *nsdC* enhanced formation of fruiting bodies (cleistothecia), implicating NsdC as a key positive regulator of sexual development. Deletion of *nsdC* also retarded vegetative growth and hyper-active asexual sporulation. Moreover, conidia were induced in liquid medium with glycerol or lactose as the sole carbon source, but not with glucose or acetate. Collectively, the results suggest that NsdC is not only necessary for sexual development but also negatively regulates asexual sporulation especially on carbon sources favoring sexual development. Over-expression of *nsdD* restored the retarded growth and conidiation in liquid medium but not the inability of fruiting body formation caused by *nsdC* deletion, which implies that NsdD coordinately joins in the control of growth and repression of asexual development by NsdC. Over-expression of *veA* or *nsdD* does not rescue the failure of fruiting body formation by nsdC deletion mutation. Furthermore, *nsdC* expression is not affected by either VeA or NsdD, and vice versa, indicating that NsdC regulates sexual development independently of VeA or NsdD.

222. Transcriptional regulation by PP1, a *STE12* homolog in *Neurospora crassa*, during germling fusion as determined via microarray profiling and ChIP- Seq

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The transcription factor *STE1*2 is involved in two distinct developmental programs of mating and filamentation in the yeast *Saccharomyces cerevisiae*. In *Neurospora crassa*, mutations in the *STE12* homolog (*pp-1*) result in strains showing reduced hyphal growth, female-sterility and defects in germling/hyphal fusion. Unlike Ste12p, PP1 contains two DNA binding domains: a homeodomain and a zinc finger domain. We used site-directed mutagenesis to study functions of these two domains in PP1. Mutations in the zinc finger region showed no adverse effects on sexual development, vegetative growth or germling fusion. However, strains containing a single mutation in the PP1 homeodomain region showed ascospore lethality and fusion deficiency. More interestingly, the effect of the mutations in *pp-1* during vegetative growth was dependent on the promoters used. Strains containing a native *pp-1* promoter driving the *pp-1* homeodomain mutation showed a similar growth phenotype to a ‹¢pp-1 strain, while this same construct with a *ccg-1* promoter showed wild type growth. To further study PP1 transcriptional regulation during germling fusion, we used microarray profiling at different time points for a wild type strain, a *pp- 1* deletion strain, and a *pp-1* homedomain mutation strain to identify fusion- related and PP1-regulated genes. By chromatin immunoprecipitation with epitope-tagged alleles of *pp-1* and subsequent Solexa sequencing, direct target genes of PP1 will be identified.

223. Developmental regulation of mating type gene *matA* in homothallic *Aspergillus nidulans*

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Mating type gene *matA* in *Aspergillus nidulans (An)* encodes an essential transcription factor that controls fruiting body development and asci differentiation during sexual development. Developmental, spatio-temporal expression of *matA* gene is critical to control different stages of sexual development in homothallic *An.* The regulation and expression of *matA* gene during sexual stage in *An* has not been fully understood. Here we demonstrate that homothallic (self- fertile) *Aspergillus nidulans,* by contrast to cross-fertile heterothallic fungi, has unique regulatory mechanisms that correctly control *matA* gene expression and mating type functions that are localized within the same nucleus. Our data show that correct expression of *matA* gene and associated molecular functions strictly depend upon genetic position and gene copy number. Duplication of the *matA* gene in the An genome results in completely barren fruiting bodies. When the duplicated *matA* copy is removed, fertile cleistothecia with wild type levels of ascospore are made. Furthermore, integration of a full length *matA* gene copy at the resident and in tandem with a *?matA* locus results in correct fruiting body development, however the ascospore number is dramatically reduced. Our data suggest new regulatory mechanism associated with mating functions in homothallic fungus.

224. Ftf2 encodes a fungal transcription factor involved in growth but not in pathogenicity

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The gene ftf2, encoding a transcription factor with a Zn(II)2-Cys6 binuclear cluster DNA- binding motif, was discovered in *Fusarium oxysporum f.sp. phaseoli* and homologs are found in other pathogenic and non-pathogenic fungi. Southern and genome analyses detected a single ftf2 copy. Previously, a multicopy gene (ftf1) was characterized that is highly homologous to ftf2. Ftf1 is only present in highly virulent strains of *F. oxysporum* and is dramatically upregulated during the early stages of the infection. To date, however, the function of the ftf2 gene product is still unknown.
Silencing of ftf2 was induced in *F. oxysporum*. *Agrobacterium tumefaciens*- mediated transfer of a hairpin-expression vector carrying fragments of the ftf2 gene allowed efficient silencing of ftf2 expression. The silenced transformants showed a reduction in their growing capacities and, possibly, a reduction in sporulation. However, no effect on pathogenicity was observed. Also, Real-time PCR analysis indicated that the expression of ftf2 is similar in all the strains and rather constant in time, suggesting a constitutive gene expression pattern for ftf2. Also, no upregulation or downregulations was observed in planta, confirming the absence of an important role for ftf2 in the infection process.
Finally, in order to shed some light on the kind of genes regulated by the ftf2 transcription factor, a SSH library was constructed. Two mRNA populations were used that were obtained from the weakly virulent wild type strain FOP-SP4 and a ftf2 silenced FOP-SP4 transformant. Preliminary analysis of the library indicates the presence of genes that are expressed in the tester strain yet absent from the driver RNA, and thus are putatively regulated by the ftf2 transcription factor.

225. Genome-wide mapping of the coactivator Ada2p yields insight into the functional roles of SAGA/ADA complex in *Candida albicans*

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SAGA/ADA coactivator complex is widely conserved throughout eukaryotes and it was shown to regulate numerous cellular processes through the coordination of histone acetylation. We investigated the multiple functions of SAGA/ADA in *C. albicans* by determining the genome-wide occupancy of Ada2p using ChIP-chip. Ada2p is recruited to 200 gene promoters involved in different stress-responses and metabolic processes. Phenotypic and transcriptomic analysis of *ada2* mutant showed that Ada2p is required for the responses to oxidative stress, as well as to treatments with tunicamycin, and fluconazole. Ada2p recruitment to the promoters of oxidative resistance genes appears mediated by the transcription factor (TF) Cap1p, and coactivator function were also established for Gal4p, which recruits Ada2p to the promoters of glycolysis and pyruvate metabolism genes. Co-occupancy of Ada2p and the drug resistance regulator Mrr1p on the promoters of core resistance genes characterizing drug resistance in clinical strains was also demonstrated. This study also arose evidence that cases of transcriptional rewiring are not restricted to TF but also to their co-activators. Indeed, while binding of Ada2p to glycolysis promoters requires the presence of Gal4p, Ada2p is not required for galactose metabolism in *C. albicans*. Role of Ada2p in acetylation and virulence has been also established.

226. Defining a mechanism of circadian output in Neurospora crassa: temporal regulation of a MAP kinase pathway.
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Our focus is to uncover mechanisms that relay temporal information from time-keeping circadian oscillators to the genes and processes that show rhythmic activity. In the filamentous fungus *Neuropora crassa*, an osmosensing p38 MAP kinase pathway (OS) is rhythmically activated and functions as an output pathway from the circadian oscillator. This rhythmic activity controls the expression of clock-controlled gene-1 (*ccg1*). Knockouts and constitutively active mutants of the response regulator-1 gene (*rrg-1*), as well as a null mutant of *os-2*, the MAP kinase, eliminate rhythms in *ccg-1*. Surprisingly, several knockout mutants in the OS pathway, namely the histidine kinases *?sln1* and *?os1* and the MAPKKK *?os-4*, do not abolish rhythmicity of *ccg-1*. These data suggest that there are multiple clock inputs conferring regulation to the OS pathway, or that other signaling components can compensate for the loss of OS pathway components. Our working model suggests an intact OS pathway is required for rhythmic output from the clock and that deficiencies in this pathway may be overcome by the two other parallel MAPK cascades. Due to high conservation of the p38 MAPK in diverse organisms, we have examined if activity of the mammalian p38 MAPK homolog is also influenced by the clock. Our data are consistent with a role for conserved MAPK signaling cascades in the mediation of circadian rhythmicity.

227. The role of TOS9 domain proteins during growth and morphogenesis in the dimorphic human pathogen *Penicillium marneffei*
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Many fungal pathogens have the capacity to undergo dimorphic switching and this capacity is tightly linked to pathogenicity. Studies in *Candida albicans* identified the *wor1*/*tos9* gene as a master regulator of white/opaque (WO) switching (Zordan *et. al.*, 2006; Huang *et. al.*, 2006; Srikantha *et. al.*, 2006) and it has been show to function in a feedback loop with a number of other transcription factors known to be involved in the WO switch (Zordan *et. al.*, 2007). Mutagenesis studies in the dimorphic pathogen Histoplasma capsulatum identified the *ryp1* gene, a homologue of WOR1, and *ryp1* mutants are unable to switch from hyphal to yeast growth (Nguyen and Sil, 2008). Two further genes (*ryp2* and *ryp3*) were found to also be required for filamentous growth and are homologues of the Velvet A family of regulatory proteins in filamentous fungi (Webster and Sil, 2008).
The only known domain of RYP1 and WOR1 is the TOS9 domain. The founding member of this family is the *Schizosaccharomyces pombe pac2* gene which is involved in the regulation of mating (Kunitomo, *et. al.*, 1995).
Like *H. capsulatum*, the dimorphic human pathogen *Penicillium marneffei* also switches from hyphal to yeast growth with the switch of temperature to 37°C but divides by fission rather than by budding. *P. marneffei* has homologues of RYP1/WOR1 and of PAC2. The genes encoding these proteins have been cloned and characterised in *P. marneffei* using deletion and overexpression strains. In addition, their role in the regulation of target genes has also been examined in order to understand how they control growth and morphogenesis.

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228. Identification and characterization of Neurospora cell fusion mutants.
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We have screened the first 81 plates of the Neurospora KO library for mutants affected in the process of anastomosis. We found about 200 such mutants in the library, but many of these mutants were not tagged by the insertion of the hygromycin gene used to create the KO library. Preliminary data suggests that there is a high level of mutagenesis that is occurring during the library construction. About 60 of the mutants have a KO tagged mutation responsible for giving the anastomosis-defective phenotype. These mutants can be roughly divided into three groups. One of these groups includes a number of genes involved in different aspects of intracellular signaling pathways. A second group includes a number of transcription factors. The third group includes a number of secreted and/or cell wall proteins that function in the cell fusion process. We have focused out attention on this third group of mutants and present evidence for the role of some of these proteins in anastomosis. Some of these proteins have GPI anchor attachments signals that would direct them into the cell wall. Many of these proteins have homologs in other filamentous fungi, but do not have known functions assigned to them.

229. Mutations to *LmIFRD* affect cell wall integrity of the ascomycete *Leptosphaeria maculans*.
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The cell wall of filamentous ascomycetes generally comprises of a central core of branched beta 1,3/1,6 glucan chains that are linked to chitin via a beta 1,4 linkage and stabilized by associated proteins. This cross-linked structure provides strength to protect the fungus from changes in the environment, but undergoes extensive remodelling especially at the growing hyphal tips. We describe a gene *LmIFRD* (*Leptosphaeria maculans* Interferon-Related Developmental Regulator) involved in the maintenance of cell wall integrity of *Leptosphaeria maculans*, the fungal pathogen that causes blackleg disease of canola.
A T-DNA mutant with an insertion in *LmIFRD* was identified through a screen for loss-of-pathogenicity mutants. The T-DNA insertion, within the third exon of the *LmIFRD* open reading frame, results in expression of a truncated transcript. To aid in the characterisation of LmIFRD function, the expression of *LmIFRD* in the wild type strain was reduced by 89% via RNAi-mediated silencing. The T-DNA mutant had increased tolerance to cell wall-disrupting compounds, such as congo red and calcofluor white, and increased tolerance to the chitin synthase inhibitor, nikkomycin Z. Conversely, the RNAi-silenced mutant displayed decreased tolerance to these compounds compared to the wild type strain. Differences in cell wall structure and/or composition were reflected by different intensities of binding of a fluorescently labelled lectin, wheat germ agglutinin, which is specific to N-acetyl glucosamine polymers. Cell walls of the T-DNA mutant compared to the wild type isolate had increases in amounts of 1,3-galactan and chitin and decreases in 1,3-glucan and 1,4-glucan, whilst increases in 1,6-glucan and 1,2,6- mannan were detected in the cell walls of the RNAi-silenced mutant. This is the first report of the monosaccharide composition and linkage analysis of cell walls for any dothideomycete.

230. Protein kinase II (CK2) mediated phosphorylation of phosducin-like protein BDM-1 affects G-beta subunit accumulation in *Cryphonectria parasitica*.
Joanna Salamon-Kozubowska, Rachel Acuna and Angus L. Dawe. Department of Biology, New Mexico State University, Las Cruces, NM 88003. *C. parasitica* is a plant pathogen and causative agent of chestnut blight disease. Genetic studies supported by completion of the genome have revealed that this fungus contains three G-alpha, one G-beta and one G-gamma subunits that have important roles in pigmentation, sporulation and virulence. Evidence from mammalian systems suggested that phosducin-like protein BDM-1 is a potential regulator of G-beta-gamma signaling and may be phosphorylated by CK2. In *C. parasitica*, deletion of either the beta subunit or BDM-1 produces identical phenotypes, including reduced accumulation of the CPG-1 alpha subunit. Additionally, the G-beta subunit is not detectable in absence of BDM-1. To investigate the role of BDM-1 in G-beta-gamma signaling, we confirmed that BDM-1 can be phosphorylated by CK2. We have identified five putative CK2 phosphorylation sites and substituted these with either alanine or aspartic acid in a FLAG-tagged BDM-1. Analysis of the mutants has revealed that CK2-mediated phosphorylation at only two or three of these sites is physiologically relevant. However, a lack of significant change in phenotype suggests that CK2 is not the only regulatory mechanism for BDM-1. Interestingly, co-expression of the BDM-1 mutants in the presence of Myc-tagged G-beta resulted in changes in growth, pigmentation and overexpressed G-beta accumulation, indicating that phosphorylation of BDM-1 by CK2 influences G-beta stability.

231. ROS, RAS-1, growth and development.
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In *Neuropspora crassa*, conidiation is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: hyphae adhesion, aerial hyphae growth and conidia development. Each transition is started by an unstable hyperoxidant state and results in growth arrest, autophagy, an antioxidant response and a dioxygen insulation process. These responses stabilize the system and, once stable, growth can start again. We hypothesized that RAS-1 acts as a switch between growth and cell differentiation. The "band" mutant (*bd*) has a dominant *ras-1* mutation that results in the alternation of growth and conidiation. *ras-1*^{bd} has an inappropriate signaling through the MAK-2 and OS-2 kinases and develops increased oxidative stress during conidiation. RAS-1 altered signaling affects both growth and cell differentiation.

232. Functional characterization of an Oospore specific gene family from the mycoparasite *Pythium oligandrum*

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The oomycete *Pythium oligandrum* is used as a biocontrol agent because of the symbioses it forms with plants, fungi, and other oomycetes. It can parasitize phytopathogenic fungi and oomycetes providing protection to crop plants. *P. oligandrum* expressed sequence tags derived from vegetative mycelia and a *P. oligandrum-P. infestans* interaction were analyzed in an attempt to find sequences that may be involved in its biotic interactions. Many sequences with similarity to previously described effectors from fungi, oomycetes, and bacteria were revealed. We also identified a family a genes in the EST libraries that encode small proteins that are tyrosine-rich (STR) with some similarity to nematode eggshell protein-encoding genes. Using a *P. oligandrum* transformation protocol, we silenced the expression of these genes by homology-dependent gene silencing. Oospores from silenced strains displayed major ultrastructural abnormalities and were sensitive to degradative enzyme treatment. The proteins were localized to the oogonial and oospore wall. We therefore suggest that these proteins are integral components of the oospore/oogonial cell wall.

233. Functional differentiation of fungal chitinases.

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Chitin is the second most abundant natural polysaccharide found in nature and it is a major component of the fungal cell wall. Chitinases are enzymes that are capable of degrading chitin directly to low molecular weight products by hydrolyzing the glycosidic bonds. Chitinases also have aggressive roles as fungal pathogenicity factors during infection of other fungi. Based on their amino acid sequence, chitinases are divided into two different glycoside hydrolase families (18 and 19) which are further subdivided into groups. Fungal chitinases belongs to glycoside hydrolase family 18. Analyses of complete fungal genome sequences have revealed a large diversity of novel types of putative chitinases. Subgroup CI and CII chitinases contain LysM peptidoglycan binding domains and chitin-binding type 1 domains. They show similarity with the yeast killer toxin from *Kluyveromyces lactis*, and are therefore believed to be involved in antagonistic interactions with other fungi. Knockout mutants of CII chitinases are constructed by homologous gene targeting in *Aspergillus nidulans*. In addition, knockout mutants of CII chitinases in *Neurospora crassa* are screened for different phenotypic characters. In another study, Green Fluorescence Protein is going to be used as a tag for CII chitinases in *A. nidulans* to study spatial and temporal expression patterns during antagonistic interactions.

234. Conidial germination and trehalose metabolism in *Aspergillus niger*

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The asexual conidia of filamentous fungi are metabolically active structures that will germinate in the right environment. We study the factors required for germination, and how it can be prevented, starting by investigating the trehalose metabolism in the common food spoilage mould *A. niger*. The disaccharide trehalose is accumulated in the conidial cell wall under development and the sugar is released during germination. It has previously been reported that trehalose protects spores from rehydration- and heat shock stress. We decided to investigate this further by creating several null mutants in the trehalose synthesis and breakdown pathways. The phenotypes are tested in respect to morphology, trehalose contents in mycelia and in conidia, germination speed and frequency, and stress tolerance. Mutants lacking trehalose were more sensitive to the weak acid preservative, benzoic acid. Moreover, several unexpected morphologies have been observed, and we have strong indications that the trehalose synthesis pathway is more complex than previously expected.

235. Activation of apoptotic cascade in response to DNA damage in *N. crassa*.

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When cells of higher eukaryotes response to DNA damage, the each cell activates several pathways containing DNA repair, cell cycle checkpoint, and apoptosis. The results of these responses determine the cell fate, survival or death. It was appeared that several apoptotic genes are conserved in a fungus, fission yeast, and they die with apoptosis in response to some cellular stresses such as exposure to ultra violet or induction of reactive oxygen species. In the case of multi-nuclear organisms such as filamentous fungi, apoptotic mechanisms may work with different means. Then, we searched apoptotic genes in a genome database of *N. crassa*, and found some conserved homologous genes: *apaf-1*, two *caspases*, *aif*, and *EndoG*. Now, strains that had been disrupted one or several genes of them were made, and analyzed. Among several mutagens and drugs that induce DNA damages tested, 4-Nitoquinolin-*N*-Oxide (4NQO) was apparent less effective to *apaf- 1*, *caspase*, or *aif-1* disruptant. The resistance to 4NQO induced by *aif-1* mutation became clearer in the presence of mutation of *mus-38* (*Rad-1* homolog). And 4NQO sensitivity of apoptotic-gene disruptants was epistatic to *mus-9* (*ATR* homolog). Moreover, we detected fragmentation both of Caspase and DNA after 4NQO treatment. These results indicate that apoptotic cascades are induced in *N. crassa* in response to DNA damages, and connected with both DNA repair and cell cycle checkpoint mechanisms.

236. The protein kinase ImeB is involved in the coordination of sexual development and mycotoxin production in *Aspergillus nidulans*.

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Yeast Ime2 and *U. maydis* Crk1 are members of a conserved protein kinase family related to MAPK. We have identified the *imeB* gene encoding an *Aspergillus nidulans* ortholog of Ime2/Crk1. *imeB* deletion strains are retarded in growth. On solid medium, *imeB* mutants produce many fertile cleistothecia in the presence of light, which normally represses development of these sexual fruiting bodies. In liquid, *imeB* strains display abnormal differentiation of sexual Hülle cells, suggesting that ImeB acts as inhibitor of sexual development. We provide evidence that ImeB and the red-light receptor FphA cooperate in light-mediated inhibition of fruiting body formation. A combined deletion of *imeB* and *fphA* results in complete loss of light response. We further show that *imeB* deletion strains fail to produce the aflatoxin precursor sterigmatocystin (ST). *stcU* mRNA is reduced to marginal levels, implying that ImeB is needed for expression of the ST gene cluster. We conclude that ImeB is required for coordinating light-controlled development and ST production. We further show that the TXY motif conserved in MAPK is essential for ImeB function. Finally, our data suggest that, within the phylum of ascomycetes, the related Ime2 and ImeB proteins have acquired opposing functions with respect to sexual development: Ime2 promotes meiosis and ascospore formation, but ImeB inhibits the sexual life cycle.

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237. Roles of two paxillin homologues in *Aspergillus nidulans*

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Paxillin is a scaffold protein localized at cell adhesion sites in mammalian cells. Through multiple domains it connects the extracellular matrix with the intracellular surface and orchestrates a multitude of proteins responsible for responses to stimuli occurring at these sites. We identified two paxillin homologues in *Aspergillus nidulans*, PaxA and PaxB, with distinct functions. PaxA was detected as a surface crescent at hyphal sites of localized polarized growth indicating a function at the hyphal tip. Deletion of *paxA* resulted in a preserved ability to grow in a polarized fashion, but with a mild disruption in the maintenance of a stable polarity axis. Unlike PaxA, PaxB was detected at septation sites indicating a function in septum formation. Deletion of *paxB* resulted in defects during constriction of the cytokinetic actin ring. In addition, analysis of double mutants indicated an interaction of PaxB with the GTPase-activating protein CenA, a homologue of centaurin. We propose that in *Aspergillus nidulans* paxillin proteins have diverged to execute different functions.

238. Characterisation of the RAM pathway in *Ustilago maydis*

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The RAM pathway (Regulation of Ace2 activity and cellular Morphology) has been characterized in different organisms and it has been shown to be involved in the regulation of essential mechanisms of cell growth. In *Saccharomyces cerevisiae*, the components of this pathway are Hym1, Nak1, Tao3, Cbk1, Mob2 and the target protein Ace2. This pathway has been shown to play important functions in cell separation and polarity maintenance in yeast, though few functions are known for this pathway in fungi. In *Ustilago maydis*, we characterized the components of the RAM pathway, in which Ukc1 is the homolog of the central protein kinase Cbk1. Cells deleted for any of these components present a defect in cell separation, morphology and are unable to infect plant maize. We have also demonstrated in this work that the avirulence of RAM- deficient strains was due to a defect in pheromone response, which is required for cells to mate. Recently, the connection between Ndr protein kinase (Cbk1 protein family) and PKA protein has been demonstrated in *Neurospora crassa*, where the inhibition of PKA activity can suppress the *cot1* mutant deleted phenotype, protein homolog to Cbk1. In *U. maydis*, PKA activity has been shown to be essential for corn smut disease and the connection between PKA and Ukc1 activity could explain the importance of the RAM pathway signalling in the virulence of *U. maydis*, and for the first time, explain its functions in morphology and polarity.

239. Aflatoxin biosynthesis is correlated to peroxisome functionality, lipid metabolism and oxidative stress in *Aspergillus flavus*

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Oxidative stress is a trigger for different metabolic events in all organisms and occurs during oxidative processes in the cell such as peroxisomal beta-oxidation of fatty acids. To study whether peroxisome functionality, oxidative stress and oxylipins formation are related to aflatoxin biosynthesis in *A.flavus* a gene encoding for a virus (*Cymbidium ringspot virus*) protein, p33, which is able to induce peroxisome proliferation, was inserted in NRRL 3357 WT strain. The peroxisome hyperproliferation in Afp33 strains was demonstrated by TEM analysis. These organelles were also labelled with a SKL (target peptide for peroxisome) - dsRED fluorescent protein. The p33-GFP was found to co-localise with the red dsRED fluorescent protein. The expression of some peroxisome functionality markers (*foxA*, *pex11*) was also monitored. The WT and the mutant strains were compared by means of phenotype microarray technique. In Afp33-dsRED strain an up-regulation of the lipid metabolism (upregulation of the TCA cycle, FFA beta-oxidation and TG accumulation) is the putative cause for the induction of a hyperoxidant status (higher ROS and oxylipins formation) and aflatoxin biosynthesis enhancement both *in vitro* and *in vivo* (maize seeds). *In silico* N_SITE analysis of the *aflR* promoter region indicates the presence of regulatory elements (RE) responsive to CREB (cAMP), SREBP-ADD1 (lipid metabolism), AP1 (human ortholog of Yap1 and ApyapA), and PPARalfa (lipid metabolism) binding factors, further stressing a close connection among the onset of oxidative stress, oxylipin formation and toxin synthesis.

240. Withdrawn

241. Construction of autonomously replicating vectors for complementation analysis of disruption mutants in a *ku70* deletion background in *Aspergillus niger*.

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Mutants with a defective Non-Homologous-End-Joining (NHEJ) pathway are a very powerful tool for fungal genetic engineering. Several reports over the last few years have shown that mutants in the NHEJ–pathway (ku70/ku80 mutants) are very efficient recipients for gene targeting and achieve homologous targeting efficiencies up to 100%. To prove that a phenotype is associated with the deletion of a certain gene, the gene of interest is transformed back to the gene deletion strain which will ectopically integrate into the genome. However, phenotype complementation becomes difficult in a *ku70* deletion background because ectopic integration frequencies are low and the gene will preferably integrate via homologous recombination, thereby replacing again the disrupted gene. One way to circumvent this problem is to clone the gene for complementation into an autonomously replicating plasmid containing a selection marker. Under selective pressure the plasmid is maintained, giving the wild type phenotype; once the selective pressure is removed, the plasmid is gradually lost and the mutant phenotype is again observed. We have constructed autonomously replicating vectors containing either the *pyrG* or Hygromycine as selection markers and a unique *NotI* site for easy cloning of complementing genes. These vectors have been successfully used for complementation analysis of gene deletion mutants in *A. niger*.

242. Cell wall chitosaccharides are essential components and exposed patterns of the phytopathogenic oomycete *Aphanomyces euteiches*

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Chitin is an essential component of the fungal cell wall, and chitooligosaccharides derived from it are signaling molecules involved in plant-microbe interactions. Oomycetes are fungal-like organisms which usually lack chitin. First study of the cell wall of the oomycete *A. euteiches*, a major legume parasite, showed the presence of 10% N- acetylglucosamine, that corresponds to amorphous chitosaccharides rather than to crystalline chitin. Two putative chitin synthase (CHS) genes were identified and full length cDNA sequences of both genes were obtained. Phylogeny analysis indicated that oomycete CHS diversification occurred before divergence of the major oomycete lineages. Remarkably, lectin labeling showed that the chitosaccharides are exposed at the cell wall surface, and study of the effect of the CHS inhibitor Nikkomycine Z demonstrated that they are involved in cell wall function. These data open new perspectives for the development of anti-oomycete drugs and further studies of the mechanisms involved in recognition of pathogenic oomycetes by host plants.

243. The mitotic NIMA kinase shows synthetic lethal interactions with genes potentially involved in septation and cell tip growth in *Aspergillus nidulans*.

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In *Aspergillus nidulans* the transition from G2 into mitosis requires the NIMA kinase. In yeast, a synthetic lethal screen using *KIN3*, the non essential NIMA orthologue, identified twelve genes, not involved with the cell cycle (S.L. McGuire, unpublished). Since *KIN3* does not have mitotic functions, if these synthetic interactions where conserved in *A. nidulans* this might reveal additional non- mitotic roles for NIMA. We therefore determined if NIMA interacts with the orthologues of the yeast genes that are synthetically lethal with *KIN3*. Of the ten orthologues identified, synthetic lethal/sick temperature sensitive interactions with *nimA7* were identified for only four, An-*swd1*, An-*vps23*, An-*vps25* and An- *ypt7*. We characterized the nuclear morphology and septation of the double mutants which revealed temperature sensitive synthetic growth defects linked to abnormal branching, septation and DNA segregation. Because we additionally discovered a defect in the tip morphology of *nimA*^{ts} cells, collectively the results indicate that NIMA plays roles in septation and tip growth. This hypothesis is strengthened by the observation that NIMA localizes to septa and cell tips (C. De-Souza, K-F. Shen and S.A. Osmani unpublished). Further characterization of these interactions will lead to a better understanding of these previously unrealized non mitotic functions of NIMA.

244. Interaction between microtubule plus end and hyphal tip cortex for polarity signaling in *Aspergillus nidulans*

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During cellular morphogenesis among the different cell types, microtubules deliver positional information to the proper site of cortical polarity. Once microtubules and their associated proteins determine the polarity site, a positive feedback loop initiates to reinforce and maintain the polarity site through rearrangement of actin cytoskeleton. To perform the function, microtubules have to search a specific site at the cortex and stop elongation once they reach the cortex. Here, we show in *Aspergillus nidulans* that the contact of microtubule plus ends with the cortex is mediated through protein-protein interaction between a processive microtubule polymerase (XMAP215, *A. nidulans* homologue AlpA), and a cortical cell end marker protein, TeaA. Although both proteins localized to the microtubule plus end during microtubule growth, AlpA-TeaA interaction was observed only after contact with the cortex. In the absence of TeaA, microtubules continued to grow after reaching the cortex. In the absence of AlpA, the microtubule array was largely affected and microtubules grew very slowly. To determine if AlpA polymerase activity is directly controlled by the interaction with TeaA, we developed an *in vitro* assay system. Our results suggest that microtubule polymerization depends on the presence of AlpA and growth is inhibited by TeaA.

245. Hyphal heterogeneity at the periphery of colonies of *Aspergillus niger*.

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Filamentous fungi, like *Aspergillus niger*, colonize substrates by means of leading hyphae. These hyphae secrete enzymes, e.g. glucoamylase, to convert polymers in the medium into small products that can be taken up to serve as nutrients. Fascinatingly, not all leading hyphae of *A. niger* secrete glucoamylase. Furthermore, they also do not express the glucoamylase gene, *glaA*, to the same extent. About half the hyphae were found to express *glaA* highly, whereas the other half expresses this gene at a low level. This was unexpected, since all hyphae were exposed to identical environmental conditions. This finding raised the question whether expression of genes encoding secreted enzymes is restricted to particular hyphae or whether each hypha expresses different enzymes. To study this, promoters of genes encoding secreted proteins and/or their regulators were fused to GFP and dTomato. Correlation of GFP and dTomato fluorescence is currently being analyzed with confocal microscopy in *A. niger* strains expressing these reporter constructs. Preliminary results show that expression of *glaA* and its regulatory gene, *amyR*, correlate strongly. In contrast, expression of *glaA* and *faeA*, the latter being regulated by XlnR, correlates weakly. These and other results suggest that secretion heterogeneity is regulated at the level of the respective transcriptional activators. This research is supported by the Dutch Technology Foundation STW, applied science division of NWO and the Technology Program of the Ministry of Economic Affairs.

246. Analyzing of COT1 interacting components in the fungus *Neurospora crassa*

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Members of the NDR serine/threonine protein kinase family play important roles in cell differentiation and polar morphogenesis. The NDR kinase COT1 of *N. crassa* is essential for hyphal elongation and to restrict excessive branch formation. *cot-1* mutants show growth defects characterized by hyperbranching and cessation of cell elongation. By using biochemical, genetic and cell biological approaches, we have established a network of COT1 interacting proteins consisting of POD6, several MOB proteins and HYM1. Based on the identical phenotypes and genetic characteristics of *cot-1* and *pod-6* mutants and by comparing this with NDR kinase networks in other organisms, we suppose that POD6 is acting as the upstream kinase and is involved in activating COT1. Furthermore, we identified MOB2A and MOB2B as COT1 interacting proteins by coimmunoprecipitation studies. Kinase assays indicate that both proteins are essential for COT1 activation. In line with these data, *mob-2a;mob-2b* double mutants display typical *cot-1/pod-6* defects. We also identified HYM1 as COT1 and POD6 interactor by coimmunoprecipitation and yeast two hybrid analyses and as COT1 regulator by COT1 activity measurements. However, in contrast to the identical phenotypes of the central complex components *cot-1, pod-6* and *mob-2a;mob- 2b, hym-1* displayed cell fusion and developmental defects, which are highly reminiscent to mutations in the MAK2 mitogen activated protein kinase cascade. Currently we are analyzing this interacting network and its potential cross-communication with other pathways in *N. crassa* in more detail and suggest a potential relevance to similar NDR signaling pathways in higher eukaryotes.

247. Identification and characterization of cell wall and secreted proteins from *Neurospora crassa*, *Candida albicans*, and *Saccharomyces cerevisiae.*

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We have used trifluoromethanesulfonic acid to disrupt glycosidic linkages in isolated cell wall and secreted protein preparations and have used LC/MS/MS to identify many of the major proteins in the cell walls of Neurospora, Candida and Saccharomyces. He have characterized Neurospora KO mutants lacking these different cell wall proteins. The mutants were obtained from the Neurospora Genome Project. We have identified a number of phenotypes associated with the loss of different cell wall proteins, highlighting the different functions that are associated with the cell wall. These phenotypes include difficulty in generating different cell types, such as fusion pegs and CATs, protoperithecia, perithecia and functional ascospores. Some mutants have affected in colonial morphologies, hyphal branching patterns, cell signaling events, and anastomosis. We find that in many cases, loss of a cell wall protein results in a cell wall that is fragile when compared to the wild-type cell wall. Examples of mutants affecting different cell wall functions are presented. The approach of isolating and characterizing cell wall mutants allows for the genetic dissection of cell wall functions.

248. Laccase and polyketide synthase affect virulence and sporulation in the corn smut pathogen *Ustilago maydis*

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Ustilago maydis, a maize pathogen, must produce teliospores to complete its life cycle. Teliosporogenesis normally occurs only *in planta*. However, deletion of *ust1*, encoding an APSES domain protein, yielded a striking phenotype including the production of highly pigmented spore-like structures in culture. Therefore, the *ust1* mutant provides a host free genetic system to begin to decipher the sporulation associated transcriptome. To this end we are utilizing microarray analysis of the *ust1* mutant for comparison with in planta sporulation. Analysis showed 36 genes upregulated 20 fold or higher in the *ust1* mutant. One of these genes is *ssp1*, previously published as specifically and highly expressed in teliospores. This confirms that the *ust1* is a good surrogate for sporulation. We have thus far focused on two of these upregulated genes, laccase and polyketide synthase. Laccase because of its role as a cell wall-associated virulence factor and melanin biosynthesis of the spore wall in *Cryptococcus neoformans*. Polyketide synthase due its requirement for virulence and T-toxin production in *Cochliobolus heterostrophus* and its involvement in melanin biosynthesis pathway of the phytopathogenic fungus *Bipolaris oryzae*. We generated a laccase deletion mutant, which exhibit delayed pathogenicity. We generated a polyketide synthase deletion mutant and our preliminary results showed it to be dramatically impaired in pathogenicity. Interestingly, galls of the polyketide synthase mutant also showed reduced teliospore production.

249. Sterol rich membrane domains support cell cell signaling and fusion in *Neurospora crassa*.

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In *N. crassa* germinating vegetative spores (conidia) show mutual attraction, directed growth towards each other and cell fusion. Earlier studies suggested that the SO protein and the MAP kinase MAK-2 are essential for signaling between the two fusion cells (Pandey et al. 2004, Fleissner et al. 2005). Both proteins are recruited to the tips of fusion germlings in a coordinated oscillating manner (Fleissner et al. unpublished) and concentrate at the fusion point once the two cells get into physical contact. Here we show that the *erg-2* mutant, whose cell membrane is ergosterol deficient, is affected in cell cell communication and fusion. Chemotropic interaction between *erg-2* cells is reduced. If two *erg-2* germlings achieve physical contact, fusion is highly impaired and the two germ tubes finally grow away from each other. Analysis of the subcellular localization of the SO protein and MAK-2 showed that recruitment of these proteins to the fusion tips is highly reduced in the *erg-2* mutant. These data suggest that sterol rich membrane domains are required for the efficient and proper localization of signaling proteins during the process of cell communication and fusion.

Pandey et al. (2004) Eukaryot. Cell 3(2): 348-358 Fleissner et al. (2005) Eukaryot. Cell 4(5): 920-930

250. Survival of *Cryptococcus neoformans* at 37°C depends on the integrity of the septin protein complex

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Surviving stress in the host is crucial for virulence of the basidiomycete *Cryptococcus neoformans*. We found that genes encoding homologues of the septin family (Cdc3, Cdc11 and Cdc12) were essential for growth at 37°C but not at 24°C. Septins are conserved, filament-forming GTPases implicated in cytokinesis, exocytosis, cell surface organization, and vesicle fusion. They assemble into non-polar filaments at the bud neck in yeast and in the cleavage furrow of *Drosophila* and mammalian cells. We found that septin Cdc10-mCherry localized to the mother-bud neck in yeast cells, and to discrete sites of the clamp connection and the bases of the emerging spores on the basidium in the dikaryotic post-mating hyphae. Crosses between cdc3delta mutants showed that septins may not be needed for cell fusion but are necessary for efficient filamentation. Hyphae formed after mating of the cdc3delta mutants lacked properly fused clamp connections and the basidia, which were rarely present, did not form spores. Thus, septins appear to be essential for the development of basidia and clamp cell fusion. Interestingly, the cdc3delta strain exhibits significantly reduced virulence in the *Galleria mellonella* model system, even at 24°C. Thus, *C. neoformans* may require properly assembled septin complexes to survive other stresses in addition to high temperature encountered during proliferation in both heterologous and mammalian hosts.

251. Distinct and overlapping functions of the Rho-like GTPases RacA and CftA in the life cycle of *Aspergillus niger*.

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In all eukaryotic cells, Rho-related GTPases (Rho, Rac and Cdc42) are involved in controlling and organizing actin cytoskeleton and thereby controlling polarized cell growth. The genome of *A. niger* revealed the presence of four Rho-like GTPases (RhoA-D) and single copy Rac1 and CDC forty-two homologs, named RacA and CftA. To asses functions to the different Rho-like GTPases, null mutants have been generated. Of all the disruptants, the *racA* mutant displayed to most severe growth phenotype and was characterized by a hyperbranching phenotype. In addition, deletion of *racA*, *cftA*, *rhoB* and *rhoD* resulted in reduced formation of conidia. In general, the growth and morphology of the different disruption strains not severely affected, which could be explained by redundancy. The overlapping function of RacA and CftA was shown by trying to obtain a *racA*, *cftA* double deletion mutant. As all primary transformants containing both the *racA* and the *cftA* deletion were all heterokaryotic we conclude that the double mutant is lethal. The phenotypic consequences of the loss of *racA* or *cftA* on the actin cytoskeleton in germinating spores have been studied in detail. The localization of actin was examined using immunofluorescence in both the wild type and the *racA* or *cftA* deletion mutants. Actin patches in the *racA* mutant showed a severe condensation at the extreme apex at the hyphal tip while both in the *cftA* mutant and wild type strain a gradient of actin patches towards the tip was observed.

252. Rho GTPase signaling in hyphal morphogenesis of *Neurospora crassa*

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Filamentous fungi, such as *Neurospora crassa*, show extremely pronounced polar growth, leading to the formation of characteristic, highly elongated hyphae. Rho GTPases are known to be decisive signaling elements required for establishment and maintenance of cell polarity in all eukaryotes. Phenotypic analysis of *N. crassa* mutants indicated that its six putative Rho GTPases, Rho1-Rho4, Cdc42 and Rac - the latter of which lacks a homologue in unicellular yeasts - are important regulators of hyphal morphogenesis, and thus, their activity must be tightly controlled. GDP-GTP exchange factors (GEFs) and GTPase activating proteins (GAPs) play pivotal roles as they influence the nucleotide binding state of their target Rho proteins, thereby switching Rho signals on or off. Comparison of GEF with Rho deletion phenotypes allowed to associate four of the seven putative GEFs to specific Rho GTPases (NCU00668-Rho1; NCU06579/NCU02131- Rho4; NCU06067-Rac/Cdc42), while deficiency in other regulators caused no obvious Rho-related phenotype. To corroborate and expand these results, we have established *in vitro* GEF activity assays, which have already confirmed three of the proposed Rho-GEF assignments, revealed one more and will be applied to the remaining candidates. Moreover, the elucidation of the *in vivo* functions of the Rho module network and its effectors by subcellular localization and interaction analysis is under way.

253. Identification of a *Cryphonectria parasitica* gene encoding RIN1, a RAS-like small G-protein

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A novel member of RAS superfamily small G-proteins was identified in the chestnut blight fungus *Cryphonectria parasitica*. One of the salient characteristics of this G- protein was that it lacked a potential lipidation site (CAAX box) which commonly found in the C-terminal region of RAS-related proteins. Also, the protein was shown to possess a Glu residue at position of 78 which is necessary for GTPase activity. From these observations, it was concluded that the protein was closely related to mammalian Rin, a calmodulin-binding small G-protein and involved in MAP kinase signaling. Thus, this protein was tentatively designated RIN1. RIN1, encoded by a gene *rin1*, was composed of 235 amino acids and found to share about 50% amino acid identity with NRas of *Pyrenophora tritici-repentis* and RAS-2 (KRas) of *Neurospora crassa*. The *gpd-1* promoter driven overexpression of RIN1 resulted in an aberrant colony morphology, lower growth rate, less conidia production and lower virulence. Mutations which could result in loss of GTPase activity and potential membrane anchoring were also introduced into RIN1. Here, the relevance of these observations to regulations of fungal morphogenesis, growth, reproduction and virulence is discussed.

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254. The cell end marker protein TeaC regulates septation in *Aspergillus nidulans*
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Polarized growth in filamentous fungi depends on the correct spatial organization of the microtubule (MT) and actin cytoskeletons. In Schizosaccharomyces pombe it was shown that the MT cytoskeleton is required for the delivery of so called cell end marker proteins e.g. Tea1 and Tea4 to the cell ends. Here they recruit several proteins required for polarized growth, one of which is a formin which catalyzes actin cable formation. Tea4 is especially necessary for New End Take Off (NETO), which is the initiation of bipolar growth after monopolar cell growth. Latest results suggest that this machinery is conserved from fission yeast to Aspergillus nidulans. Here, we have characterized, TeaC,a putative homologue of Tea4. Sequence similarity between TeaC and Tea4 is only 12.5 %, but they both share a SH3 domain in the N-terminal region. Deletion of teaC led to an increase of the number of septa and abnormal positioning of septa. The protein localized to hyphal tips and at forming septa. TeaC interacted with the cell end marker protein TeaA (Tea1) at hyphal tips and with the formin SepA at hypal tips and at septa. Interestingly, overexpression of teaC repressed septation and caused the abnormal swelling of conidia. Those results suggest here the cell end marker protein TeaC regulates septation in A.nidulans.

255. Functional analysis of the small GTPase RAC-1 of *Neurospora crassa*.
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Small GTPases of the Rho family are conserved proteins which function as molecular switches that regulate diverse cellular functions including the establishment and maintenance of cell polarity. While Rho GTPases are inactive in their GDP-bound forms, GTP-bound forms of these GTPases are active and able to activate downstream effector proteins to perform their cellular functions. It is well established that the introduction of certain point mutations render Rho GTPases constitutively active or inactive. To investigate roles of the Rho family GTPase, RAC-1, in hyphal morphogenesis of *Neurospora crassa*, we produced strains that over-express constitutively active (CA-) or constitutively inactive (CN-) RAC-1. While overexpression of CN-RAC-1 resulted in no remarkable phenotypes compared to the wild-type strain, overexpression of CA- RAC-1 caused reduced conidium formation, a moderate increase in branch formation, and swelling of germ tubes and hyphae. These results are consistent with excess activation of RAC-1 causing aberrant hyphal polarization and morphogenesis. Overexpression of wild-type RAC-1 resulted in similar but much less pronounced effects. GFP-fused CA-RAC-1 uniformly localized to the plasma membrane of swollen hyphae, whereas it showed pronounced localization at tips of hyphae and branches that were undergoing polarized growth. GFP-fused wild-type RAC-1 also localized to hyphal tips, indicating that RAC-1 is recruited to sites of active growth. Taken together, our results suggest that spatially localized activation of RAC-1 at the cell cortex is important for the establishment and maintenance of polarized hyphal growth.

256. Common molecular mechanisms underlay appressorium- hyphopodium differentiation and invasive growth habit in *Magnaporthe oryzae*
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Magnaporthe oryzae can colonise aerial and underground plant organs with different infection mechanisms. *M. oryzae* produces melanised appressoria to infect leaves and also infects roots by mean of hyphopodia. The PKM1 pathway regulates subsequent invasive growth during both leaf and root infection. The cell biology and dissection of genetic components during *M. oryzae* appressorium development have been facilitated by plastic surfaces which induce appressoria. Hyphopodia-like structures and an invasive growth habit can also be induced on hydrophilic polystyrene. Changes in fungal cell wall structure accompanying this invasive-like growth are seen, in particular, in the chitin/chitosan ratio and glucosyl and mannosyl residues. The PMK1 pathway and the chitin-binding protein CBP1 are required for this invasive growth habit. The expression of ACE1(p)::SGFP and PLS1(p)::SGFP on leaves and roots suggests that appressoria and hyphopodia share common molecular mechanisms to penetrate host cells; lack of expression of GAS1(p)::SGFP and GAS2(p)::SGFP on roots reveals functional differences between these two structures. Plant infection tests with the mutants showing altered growth on hydrophilic polystyrene confirmed their inability to colonise plant tissues. These studies suggest that *M. oryzae* invasive growth differentiation is induced exclusively by surface recognition and does not specifically require host factors.

257. The role of APC activators Cdc20p and Cdh1p in regulating mitosis and morphogenesis.
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Candida albicans is an important fungal pathogen of humans, and its ability to switch between different cell types is critical for virulence. We previously demonstrated that depletion of the polo-like kinase Cdc5p blocked mitosis and induced polarized growth of the yeast bud that was partially dependent on the spindle checkpoint factor Bub2p. In order to elucidate how mitotic progression and spindle checkpoints are linked to morphogenesis, we investigated the function of the Anaphase Promoting Complex (APC) activators Cdc20p and Cdh1p, which are targets of the spindle checkpoint and important regulators of mitotic progression in most systems. Both factors were important for mitosis, but influenced morphogenesis, however deletion of *CDH1* resulted in a pleiotropic phenotype, including some enlarged yeast cells, while absence of Cdc20p resulted in polarized growth similar to Cdc5p-depleted cells. In contrast, absence of Cdh1p or Cdc20p resulted in small cells or large doublets, respectively, in *S. cerevisiae*. Overexpression of *CDC20* in Cdc5p-depleted cells partially suppressed the cell cycle defect, suggesting that Cdc20p may lie downstream of Cdc5p. In contrast, deletion of *CDH1* had no effect on Cdc5p-dependent polarization. While Cdc20p and Cdh1p were not required for serum-induced hyphal growth, cells lacking Cdh1p showed increased agar invasion, suggesting a complex role for this factor in regulating polarized growth. Thus, Cdc20p and Cdh1p play important roles in regulating mitosis and morphogenesis in *C. albicans*, but in a different manner than their homologues in *S. cerevisiae*. Finally, Mad2p was not required for Cdc5p-dependent polarized growth, suggesting that Bub2p is the major contributing checkpoint factor. Overall the results extend our knowledge of the regulatory circuit governing mitosis in *C. albicans* and the potential pathway underlying checkpoint- activated polarized growth.

258. Overexpression of mating-related genes restores mating ability in a non-mating strain of *Histoplasma capsulatum*

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Sexual recombination can benefit microorganisms adapting to adverse environmental conditions. Sexual recombination is also a useful genetic tool in organisms with characterized sexual cycles. *H. capsulatum (Hc)* is a dimorphic, pathogenic fungus, with a mycelial-phase sexual cycle. However, for unknown reasons, strains of *Hc* lose mating ability after several months in culture. Consequently, established lab strains of *Hc* are unable to mate. Here, we report the generation of a mating- competent strain, UC1. UC1 forms cleistothecia 4-5 weeks after co-incubation with the clinical isolate UH3. UC1 was generated by agrobacterium transformation of G217B, a non-mating lab strain, with pCB301-HYG-GFP containing the *Hc* yeast phase- specific calcium binding protein promoter regulating the green fluorescent protein geneand the hygromycin phosphotransferase (*hph*) gene, governed by the *Aspergillus nidulans* GPD promoter, flanked by loxP sites. Removal of *hph* did not abolish mating ability. Previously, pCB301-HYG-GFP was found integrated upstream of putative open reading frame HCAG 08014. RNA levels of HCAG 08014 were increased in UC1 compared to G217B in yeast phase organisms; however was not expressed in mycelial phase organisms. RNA levels of several mating-related genes, including putative alpha pheromone, "a" pheromone receptor, and MAT1-1-1 transcription factor were increased in UC1 compared to G217B. Further studies will determine whether these effects are due to the site of vector integration, or to elements on the vector itself, and the results of these studies may be used to generate mating-competent lab strains of *Hc* for general use.

259. A novel, microtubule dependent role for a formin in the filamentous fungus *Ashbya gossypii*.

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The assembly and organization of the actin cytoskeleton is fundamental for polar growth in many organisms including filamentous fungi. Key regulators of these processes are the Formin proteins. They contain characteristic sequence motifs termed formin homology domains that are important for subcellular localization and elongation of actin filaments. We show here that this is also true for the Formin from *Ashbya gossypii*. Mutation of *AgBNR2* results in an instable growth axis and frequent lyses of the tip. Since this phenotype is similar to hyphal growth after latrunculin treatment this suggests a role in actin regulation for *AgBNR2*. In agreement with this, we show that *Ag*Bnr2 is able to bind and polymerize actin. To our surprise a fusion of *Ag*Bnr2to GFP did not only localize to the tips of hyphae but also gave a punctuate pattern throughout the whole hyphae. We were able to show that these dots are identical with the spindle pole body (SPB) of the fungal nuclei. In addition we could identify a SPB-component as binding partner of *Ag*Bnr2 and we were able to map the binding motif in *Ag*Bnr2. Furthermore microtubule- binding and microtubule-stability assays suggest a direct role for *Ag*Bnr2 in regulation of microtubule dynamics in a way that is contrary to the microtubule stabilization known from mammalian formins. In conclusion our results suggest a dual role for the formin *Ag*Bnr2, with only one of its functions being related to actin and the other suggesting an involvement of *Ag*Bnr2 in the dynamics of nuclear migration via multiple microtubule interactions.

260. A screen of Neurospora knockout mutants for growth rate dependent branching.

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The spatial distribution of branch formation in wild-type Neurospora has been shown to remain constant at different growth rates. A previous survey of classical Neurospora mutants, however, yielded a collection of strains for which slow growth acted as an environmental suppressor of hyperbranching. This study repeats that survey using mutant strains from the Neurospora knockout library. Most of the mutations identified by the screen are not in genes identified as being fungal specific but serve other (presumably non-branching) roles in other organisms. Some of the genes identified are found only in lower organisms (fungi and bacteria). Only one of the knockout mutants identified as displaying growth rate sensitive branching is apparently fungal specific. The gene functions highlighted by this screen are diverse with several emerging themes including: ubiquitin-binding proteins, peroxisome-associated genes and several presumed kinases.

261. Signatures of adaptive evolution in centromere proteins of filamentous fungi.

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Centromere foundation proteins are functionally conserved but show sequence diversity. We studied two such proteins: CenH3 (CENP-A), a centromere-specific variant of histone H3, and CKP-1 ("Centromere and Kinetochore Protein-1"; CENP-C), a DNA-binding protein that interacts with CenH3 and inner kinetochore proteins. To explain the accelerated evolution of centromeric DNA and DNA-binding domains, co-evolution by adaptive evolution via "meiotic drive" mechanisms has been proposed in organisms with specialized female meiosis. Here we investigated if positive selection acts on CenH3 and CKP-1 in filamentous fungi, organisms without sex-specific meiosis. We analyzed available CenH3 and CKP-1 sequences from fungi, and sequenced numerous alleles from *Neurospora* and *Fusarium* for inter- and intraspecies sequence analysis. To find regions under positive (Ka/Ks >1) and negative (Ka/Ks <1) selection we used the MEGA and DNASP program packages. We found that: (1) a short motif in the center of CKP-1 appears under positive selection in filamentous fungi; (2) the N-terminal region of CenH3 appears under positive selection in *Neurospora* and *Fusarium*; and (3) the C-termini of CenH3 and CKP-1 are under strong negative selection. Motifs under positive selection suggest specific interactions between CenH3 and CKP-1 or between these proteins and centromeric DNA, whereas the conserved C- termini may interact with invariable nucleosome or kinetochore components. To test these hypotheses, we are expressing CenH3 from various species in an *N. crassa* CenH3 deletion mutant, swapping domains of CenH3 between *Neurospora* species, and testing site-directed mutations to define regions that are essential for normal centromere function.

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262. Characterization of the *Saccharomyces cerevisiae NDT80* homologs in *Neurospora crassa* reveals overlap between heterokaryon incompatibility and sexual development pathways

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Neurospora crassa contains 11 heterokaryon (*het*) loci that function to prevent heterokaryon formation between strains of different genotypes. Strains differing at any *het* locus are incompatible and exhibit cell death, lack of conidiation and reduced growth rate. Mutations in *vib-1* suppress heterokaryon incompatibility (HI), and two *vib-1* paralogs are encoded in the *N. crassa* genome (NCU09915, NCU04729); all three of these genes are homologous to the *S. cerevisiae* transcription factor *NDT80*. Ndt80p controls the expression of middle meiotic genes and homozygous deletion crosses are Spo-. Though the *vib-1* paralogs are not involved in HI, deltaNCU09915 strains were female sterile. These data suggested that NCU09915 may encode an *NDT80* ortholog. However, homozygous deltaNCU09915 crosses could be complemented using a mating type mutant as a "helper" strain. Thus, none of the *NDT80* homologs in *N. crassa* are required for meiosis. In *S. cerevisiae*, Ime2p is a protein kinase that regulates *NDT80* expression and activity. However, Q-RT-PCR in *N. crassa* revealed that NCU09915 is not regulated by the *IME2* homolog in *N. crassa* (NCU01498; ime2-like). Thus, it appears that *N. crassa* regulates entry into meiosis differently than *S. cerevisiae*. Surprisingly, we found that a deletion mutation in *ime-2* suppressed the *vib-1* mutant phenotype during HI. delta-*ime-2* delta-*vib-1* strains have a phenotype similar to WT incompatible heterokaryons. Thus, *ime-2* is likely an additional component of the HI pathway in *N. crassa*.

263. Two Golgi apparatus COG proteins are important to polarity establishment and maintenance in *Aspergillus nidulans.*

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*swoP*1 (swollen cell) and *podB*1 (polarity defective) mutations in *Aspergillus nidulans* interfere with establishment and maintenance of polarity. At restrictive temperatures, conidia of *swoP*1 may swell to approximately 1.5 times the normal diameter, produce abnormally wide hyphae and/or establish multiple points of polarity, which grow isotropically before arrest. Conidia of *podB*1 never establish polarity at restrictive temperatures. Cell walls of both strains are as thick as 1 μm (TEM) compared to ca. 0.04 μm at 28C, and the cytoplasm contains numerous irregular membrane structures. Genes complementing the mutations of *swoP*1 and *podB*1 have strong sequence homology to COG4 (AN7462) and COG2 (AN8226), respectively. Sequencing of the respective loci reveals point mutations causing truncations near the C-terminus. In mammals and yeast, COG2 and COG4 are part of a multi-protein structure called the COG (conserved oligomeric Golgi) complex associated with retrograde transport within the Golgi apparatus. To provide evidence for a COG function of AN7462 and AN8226, we used a high-copy AMA1 plasmid to overexpress the COG homologues of *A. nidulans* COG1-4, COG6, COG7, as well as the functionally-related homologues YPT1 (Rab GTPase) and HOC1 (mannosyl transferase). High copy expression of COG2 corrected the *swoP*1 phenotype while the remaining proteins did not. High copy expression of COG3 and COG4 corrected the *podB*1 phenotype while the remaining proteins did not. Collectively, these results support a conclusion that the SwoP and PodB proteins function in a common complex including a predicted COG3, which is consistent with the structure of the "A lobe" of yeast and mammalian COG models.

264. Whole genome analysis of the *Aspergillus nidulans* kinesins.

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Kinesins are microtubule dependent motor proteins that are essential for many cellular functions such as intracellular transport and mitotic process. In the genome of *Aspergillus nidulans*, there are11 (putative) kinesin genes. We examined the role of these kinesins in the cell by deleting these genes and by observing fluorescently tagged products of these genes in living cells. One of the kinesin genes, *bim*C, is known to be essential for growth. We disrupted each one of remaining 10 kinesin genes. Although some of the disruptants exhibited relatively minor defects in growth, all of these disruptants were viable. This result indicated that the function of these kinesins are not mutually exclusive, rather, it is likely that the functions overlap. Live imaging of fluorescently labeled kinesins revealed the localizations of each kinesin in the course of cell growth. A group of kinesins exhibited rapid translocations along the cytoplasmic microtubules indicating this group of kinesins are involved in intracellular transport. Another group of kinesins localized in the nucleoplasm either throughout the cell cycle or in a cell cycle specific manner. Two of kinesins localized at the site of septum formation indicating a novel role of microtubule motor proteins in cytokinesis. Supported by NIH GM031837 and grant in aide from JSPS.

265. mRNA trafficking during pathogenic development of *Ustilago maydis*

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Cytoskeletal transport promotes polar growth in filamentous fungi. In *Ustilago maydis*, the RNA-binding protein Rrm4 shuttles along microtubules and is crucial for establishing the polarity axis in infectious filaments. Mutations in the RNA-binding domain cause loss of function. However, it is unclear which RNAs are bound and transported. Here, we applied *in vivo* RNA binding studies and RNA live imaging to determine the molecular function of Rrm4. This new combination revealed that Rrm4 mediates microtubule-dependent transport of distinct mRNAs encoding e.g. the ubiquitin fusion protein Ubi1 and the small G protein Rho3. These transcripts accumulate in ribonucleoprotein particles (mRNPs) that move bidirectionally along microtubules and co-localise with Rrm4. Importantly, the 3' UTR of *ubi1* containing a CA-rich binding site functions as zipcode during mRNA transport. Loss of Rrm4 leads to a drastic reduction of motile mRNPs and defects in polar growth suggesting that mRNP trafficking is required for polarity. This is the first example of microtubule-dependent mRNA transport in fungi and our data provide evidence that fundamental principles are conserved among fungi, plants, and animals.

266. Centromeric regions of *Neurospora crassa* are composed of heterochromatin.

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Centromeres serve as platforms for the assembly of kinetochores and are essential for nuclear division. While pericentric heterochromatin has been documented in many eukaryotes, recent studies suggest that centromeric core chromatin is free from silencing modifications. We generated GFP and FLAG fusions to the centromere proteins CenH3 (CENP-A) and CKP-1 (CENP-C) and identified Neurospora centromeres by ChIP- sequencing. On each chromosome we found a ~100 to 200 kb region that overlaps with centromeric regions previously predicted by genetic mapping, DNA composition and presence of transposon relics. We asked which histone modifications are required for normal centromere function in Neurospora. Surprisingly, we found extensive co- localization of CenH3, CKP-1 and histone H3 K9 trimethylation ($H3K9^{Me3}$). In contrast, $H3K4^{Me2}$, which has been found at the cores of plant, fission yeast, Drosophila and mammalian centromeres, was not enriched at Neurospora centromeres. DNA methylation, which typically overlaps with Neurospora $H3K9^{Me3}$, was most pronounced at the centromere periphery but otherwise overlapped little with the distribution of centromere proteins. Mutation of dim-5, which encodes the predominant H3 K9 methyltransferase, resulted in loss of $H3K9^{Me3}$ from the centromeres. By region-specific ChIP-PCR we observed concomitant loss of CenH3-GFP binding from all but the core of two centromeres that we investigated in greater detail. Similarly, CenH3-GFP was retained only at the core in the absence of HP1, the chromodomain protein that binds to $H3K9^{Me3}$. We show that eukaryotes make use of different strategies to maintain centromeres and suggest a model in which centromere proteins nucleate at the core but require DIM-5 and HP1 for spreading and maintenance of a normal centromere.

267. Vesicle trafficking via the Spitzenkörper during hyphal tip growth in *Rhizoctonia solani*.

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Growing hyphae of *Rhizoctonia solani* were stained with the endocytotic marker dye FM4-64 and imaged by confocal microscopy. The kinetics of staining were measured in the plasma membrane, apical cytoplasm and the Spitzenkörper (Spk) of hyphal tips. Staining of the plasma membrane was followed by labeling of organelles (after ~1 min), and finally staining of the Spk (after ~ 2 min). Thus, FM4-64 is being recycled back to the plasma membrane via the Spk. Fluorescence recovery after photobleaching (FRAP) of the stained Spk demonstrated the vectorial flow of secretory vesicles from the apical cytoplasm to the Spk. The flux of vesicles through the apical cytoplasm to the Spk was mathematically modelled in a two-compartment model using measurements obtained from the kinetics of organelle staining in the hyphal tip. In this way we estimated the turnover time of the vesicles of the Spk to be 1.3-2.5 min. The calculation of the flux of vesicles through the Spk was based on estimates of the number of secretory vesicles within the Spk. The number of secretory vesicles that would be necessary to fuse with the apical plasma membrane to maintain the hyphal extension rates was similar as the calculated flux. Our quantitative results of the flux of vesicles through the Spk suggest that membrane retrieval via endocytosis is not as significant as previously suggested.

268. From cellular architecture of *Aspergillus niger* to communication networks

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Aspergillus niger is widely used for industrial production of proteins and bulk chemicals, due to its ability to secrete large amounts of proteins and metabolites into the medium. However, its mycelial morphology affects and limits the productivity of industrial processes. Therefore, much more basic knowledge is required to get a deeper insight into the molecular networks regulating the cellular architecture of *A. niger*. Coordinated control of hyphal elongation and branching is essential for sustaining mycelial growth of filamentous fungi. Although some knowledge on polarity control has been accumulated over the past years, the mechanisms underlying polarity establishment and maintenance still remain poorly understood. The temperature-sensitive hyperbranching mutant *ramosa-1* serves as an excellent model system to study critical steps of polarity control in *A. niger*, as polarity establishment and maintenance of new polarity axes can easily be induced or repressed in this strain. Identification of the genetic locus affected in *ramosa-1* revealed that a single amino acid exchange in the RmsA protein, a functional homolog of the TORC2 component Avo1p/Sin1, is responsible for increased branching. This finding suggests that TORC2 is involved in coordination of cell polarisation in *A. niger*. In order to get first insights into the signalling machinery controlling and regulating cell polarity, we used the model system *ramosa-1* to obtain a transcriptomic fingerprint of apical branching. This fingerprint indicates that several signal transduction pathways, including TORC2, phospholipid, calcium and cell wall integrity signalling, concertedly act to control apical branching in *A. niger*.

269. Microtubule cytoskeleton in the filamentous fungus *Ashbya gossypii*: organization and role in nuclear migration

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Nuclear migration is important for the normal growth and development of eukaryotes including filamentous fungi. In *Ashbya gossypii*, nuclear migration includes oscillatory movements along the hyphal growth axis and nuclear bypassing events. However, the molecular mechanisms underlying the different aspects of nuclear migration are still poorly understood and the intrinsic capacity of nuclei to sense cell polarity and move towards the hyphal tips has still to be determined. We will present pictures and movies of the MT cytoskeleton in *A. gossypii* obtained by immunostaining and by visualizing GFP labeled microtubules. We will also show the roles of MT-binding proteins in building and maintaining the MT cytoskeleton and their importance for nuclear migration. The main focus will be on the MT plus-tip binding protein Bik1, the dynein and the dynactin subunit Jnm1. The phenotypes observed in the absence of those proteins will be presented as well as the localization of Bik1 and dynein. Modifying the dynamics of cytoplasmic MTs by either deleting Bik1 or dynein/dynactin or treating cells with MT-depolymerizing drugs strongly affected nuclear migration in *A. gossypii*. We could also show that in contrast to other filamentous fungi, cytoplasmic MTs do not directly contribute to hyphal growth.

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270. Two *CDC42* Paralogs Play Distinct and Overlapping Roles in Morphogenesis and Pathogenicity in *Cryptococcus neoformans*.
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In the human host environment, the fungal pathogen *Cryptococcus neoformans* must maintain tightly regulated morphogenesis events under stress conditions, including host physiological temperature (37 deg C). We have previously shown that *RAS1* is a major regulator of polarity and morphogenesis in *C. neoformans*, and that this cascade is mediated via RHO-GTPases. Cdc42 is a highly conserved Rho family GTPase which in fungi as diverse as *Saccharomyces cerevisiae* and *Penicillium marneffei* is essential to the establishment and maintenance of polarity. Unlike other eukaryotes, the *C. neoformans* genome encodes two highly related *CDC42* paralogs. Here we further explore the individual contributions of these Rho-GTPases to actin polarization, fungal cell budding, and pathogenicity. Using gene disruption, we have generated knockouts for both paralogs singly and in combination. In this work we show that both paralogs are functional and play distinct and overlapping roles in polarity and resistance to high temperature. Further, we begin to explore the contributions of these paralogs to actin polarization, septin organization, and growth at physiological conditions.

271. Role of the peroxisome protein import complex -the importomer- in the sexual development of *Podospora anserina*.
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Peroxisome biogenesis relies on two known peroxisome-matrix protein import pathways, which are mediated by the receptors PEX5 and PEX7 and that converge at the importomer, a peroxisome membrane complex formed by the docking and the RING- finger subcomplexes and required for protein translocation. We have previously shown that in *P. anserina* transition to meiosis requires the RING-finger peroxin PEX2 but not PEX5 and PEX7. We show now that the three RING-finger peroxins, PEX2, PEX10 and PEX12, are equally required for the import of peroxisome matrix proteins and during sexual development: in their absence, dikaryotic cells are blocked before karyogamy. In contrast, lack of the docking complex peroxins PEX14, PEX14/17 and PEX13 differently affects peroxisome assembly and development. PEX14 is dispensable for peroxisome protein import at specific developmental stages, where its role could possibly be fulfilled by a PEX14/17-like protein. However, a cell-type specific protein import still occurs in absence of these two peroxins, and meiosis is induced. In contrast, no peroxisome protein import is detected in absence of PEX13, whose progression from karyogamy to meiosis is also blocked. Our results suggest a complex developmental regulation of peroxisome protein import, which could be involved in regulating meiocyte differentiation in *P. anserina*. We acknowledge ELA and ANR for funding.

272. Roles of *Aspergillus nidulans ugmA* (UDP-galactopyranose mutase) in colony growth, hyphal morphogensis, and conidiation.
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Galactofuranose (Gal*f*) is found in the cell walls of many fungi including *Aspergillus fumigatus*, currently the most prevalent opportunistic fungal pathogen in developed countries, and *A. nidulans*, a tractable model system. UDP galactopyranose mutase (UGM) converts UDP-galactopyranose into UDP-Gal*f* prior to incorporation into the fungal wall. We deleted the single-copy UGM sequence (AN3112.4, which we call *ugmA*) from an *A. nidulans nkuAdelta* strain, creating *ugmAdelta*. *ugmAdelta* strains produced viable conidia, showing that *ugmA* is not essential. *ugmAdelta* segregated independently in meiotic progeny. However, *ugmAdelta* strains had compact colonial growth, which was associated with substantially delayed and abnormal conidiation. Compared to a wildtype morphology strain, *ugmAdelta* strains had aberrant hyphal morphology, producing wide, uneven, highly branched hyphae, with thick, relatively electron-dense walls as visualized by transmission electron microscopy. These effects were partially remediated by growth on high osmolarity medium, or on medium containing 10 µg/mL Calcofluor, consistent with Gal*f* being important in cell wall structure and/or function. We have been extending this characterization using a variety of high spatial resolution spectroscopic methods. This project is supported by NSERC (KMG and SGWK), CIHR (KMG) and CIHR-RPP (SGWK).

273. The structure of organelles and the distribution of calcium transporters in *Neurospora crassa*.
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Calcium is an important signaling molecule, hypothesized to play a major role in polar growth of fungal hyphae. Analysis of the genome indicates *N. crassa* has genes for at least 5 major calcium transporters. These are an H+/Ca++ exchanger, named *cax*, and 4 Ca^{++}-pumping ATPases, named *nca-1, nca-2, nca- 3*, and *pmr*. We are investigating the role of these proteins in regulating intracellular levels of calcium. The phenotypes of mutant strains lacking transporters have been characterized and each has been tagged with GFP and/or dsRED. Because the structure and abundance of organelles is poorly described in *N. crassa* we examined the location of organellar marker proteins tagged with GFP and dsRED. The results show that the organelles, especially the vacuole, have different structure and abundance in different regions of the hypha. The large, fast growing hyphae of *N. crassa* permit a striking visualization of intracellular structure. Ca-transporters appear in the plasma membrane, the vacuole, the Golgi and the ER/nuclear envelope. Near the hyphal tip we also observed an organelle not previously described that is made visible with tagged vacuolar ATPase or the *cax* transporter.

274. Genetic analysis of CHK1 and CHK2 homologue revealed a unique cross talk between ATM and ATR pathways in *Neurospora crassa*
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DNA damage checkpoint is an important mechanism for organisms to maintain genome integrity. In *Neurospora crassa*, *mus-9* and *mus-21* are homologues of *ATR* and *ATM*, respectively, which are pivotal factors of DNA damage checkpoint in mammals. A *N. crassa* clock gene *prd-4* has been identified as a *CHK2* homologue. In this study, we identified another *CHK2* homologue and one *CHK1* homologue from the *N. crassa* genome database. As disruption of these genes affected mutagen tolerance, we named them *mus-59* and *mus-58*, respectively. The *mus-58* mutant was sensitive to hydroxyurea (HU), but the *mus-59* and *prd-4* mutants showed the same HU sensitivity as that of the wild-type strain. This indicates the possibility that MUS-58 is involved in replication checkpoint and stabilization of stalled forks like mammalian CHK1. Phosphorylation of MUS-58 and MUS-59 was observed in the wild-type strain in response to mutagen treatments. Genetic relationships between those three genes and *mus-9* or *mus-21* indicated that the *mus-9* mutation was epistatic to *mus-58*, and *mus-21* was epistatic to *prd-4*. These relationships correspond to two signal pathways, ATR-CHK1 and ATM-CHK2 that have been established in mammalian cells. However, both the *mus-9 mus-59* and *mus- 21 mus-58* double mutants showed an intermediate level between the two parental strains for DNA damage sensitivity. Our findings suggest that the DNA damage checkpoint of *N. crassa* is controlled by unique mechanisms.

275. Roles of the divergent Protein Kinase A isoforms in the growth and stress response of *Aspergillus fumigatus*
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The cAMP-dependent Protein Kinase (PKA) pathway regulates a variety of fungal developmental and stress related pathways. The opportunistic mould pathogen *Aspergillus fumigatus* contains two divergently related catalytic subunits, PkaC1 and PkaC2. A *pkaC1* deletion mutant has been reported to have defects in radial growth, asexual development, and virulence. In contrast, PkaC2 has not been described. Therefore, our studies focus on further characterizing PKA signaling in growth and stress response pathways in addition to describing potential roles for PkaC2 in these processes. Although RT-PCR analysis revealed that *pkaC2* is expressed in *A. fumigatus*, deletion of the gene did not result in detectable germination or growth defects. However, overexpression of *pkaC2* in the *pkaC1* deletion mutant led to a partial complementation of the reduced growth phenotype. Furthermore, *pkaC2* overexpression increased conidiation in the *pkaC1* mutant. With regards to stress related responses, hyphae of the *pkaC1* deletion strain were hypersensitive to hydrogen peroxide exposure as well as cell wall inhibiting agents Congo Red, SDS, and fungin. Overexpression of *pkaC2* did not significantly affect either of these susceptibility phenotypes. Interestingly, the *pkaC2* deletion strain was hypersensitive to the calcineurin inhibitors cyclosporine A and FK-506, suggesting there may be an interaction between these signaling pathways in *A. fumigatus*.

276. Mechanisms of Flo11-dependent adhesion and morphogenesis in *S. cerevisiae*.
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The cell wall protein Flo11 governs adhesion and is an important determinant of cellular morphogenesis in *S. cerevisiae*. In various strains of yeast, Flo11 may be required for flocculation, invasion, pseudohyphae, biofilms, microbial mats, and unusual colony morphologies. We find, however, that only a subset of these phenotypes are displayed by each strain. In strain Sigma 1278b, for example, Flo11 is required for agar invasion but this strain does not flocculate. Strain *S. cerevisiae var. diastaticus*, on the other hand, requires *FLO11* for flocculation but does not invade agar. We are using these differences in strain-specific Flo11 phenotypes to investigate the mechanisms by which this adhesin determines morphology. We have completed the sequence of the *FLO11* gene from strains SK1 and diastaticus and compared them to the published sequences from strains S288c and Sigma 1278b. We find substantial differences in the sequences of the gene among these strains, particularly in the central domain which encodes tandem repeats. These repeats have been shown by us and others to be involved in adhesion. To test the importance of these variations on Flo11- dependent phenotypes we carried out gene swap experiments in which we replaced the *FLO11* gene of one strain with that of another. Most of these experiments revealed surprisingly modest effects of DNA sequence variation on phenotype, suggesting that other factors influence Flo11-dependent adhesion and morphogenesis.

277. The *Aspergillus nidulans* cell end marker TeaA controls the processive microtubule polymerase XMAP215 and inhibits microtubule growth at the cortex
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During cellular morphogenesis among the different cell types, microtubules deliver positional information to the proper site of cortical polarity. Once microtubules and their associated proteins determine the polarity site, a positive feedback loop initiates to reinforce and maintain the polarity site through rearrangement of actin cytoskeleton. To perform the function, microtubules have to search a specific site at the cortex and stop elongation once they reach the cortex. Here, we show in *Aspergillus nidulans* that the contact of microtubule plus ends with the cortex is mediated through protein-protein interaction between a processive microtubule polymerase (XMAP215, *A. nidulans* homologue AlpA), and a cortical cell end marker protein, TeaA. Although both proteins localized to the microtubule plus end during microtubule growth, AlpA-TeaA interaction was observed only after contact with the cortex. In the absence of TeaA, microtubules continued to grow after reaching the cortex. In the absence of AlpA, the microtubule array was largely affected and microtubules grew very slowly. To determine if AlpA polymerase activity is directly controlled by the interaction with TeaA, we developed an *in vitro* assay system. Our results suggest that microtubule polymerization depends on the presence of AlpA and growth is inhibited by TeaA.

278.Withdrawn

279. The role of microtubule organizing centers for nuclear dynamics in the multinucleate filamentous fungus *Ashbya gossypii*
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Nuclear migration is important for normal growth and development of all eukaryotes including filamentous fungi. Among those, *Ashbya gossypii* is a particularly attractive organism to study nuclear distribution in multi-nucleated hyphae. Upon hyphal extension, nuclei migrate toward the growing tip, showing not only long-range migration but also extensive oscillations and by-passing of one another (Alberti-Segui et al. 2001) To better understand the role of the microtubule cytoskeleton in *A. gossypii* nuclear positioning and dynamics, we examined the structure and localization of its microtubule organizing centers (MTOCs) by EM and live cell imaging. We found no evidence of non- nuclear MTOCs by either method. Instead, we demonstrate that the multi-laminar *A. gossypii* SPB is embedded in the nuclear envelope throughout nuclear division and nucleates two types of cytoplasmic microtubules as well as nuclear microtubules. Analysis of *A. gossypii* SPB mutants showed that the outer plaque plays an important role in cytoplasmic microtubule nucleation and nuclear migration. Our data suggests that this substructure and the cytosplasmic microtubules it nucleates account for the complexity of nuclear dynamics in *A. gossypii*.

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280. The *grv1* gene involved in gravitropic response of *Coprinopsis cinerea* encodes a putative anion:cation sympoter belonging to the major facilitator superfamily.

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The fruiting-body stipe of the mushroom *Coprinopsis cinerea* exhibits strong negative gravitropic response as it elongates during the final phase of development (fruiting-body maturation). We identified a *C. cinerea* mutant defective in the gravitropic response of the stipe after REMI mutagenesis. Following a plasmid rescue, we cloned the *grv1* gene, a mutation of which is responsible for the phenotype, as a genomic fragment that rescues the mutation. Sequencing of the genomic DNA, together with 3 f- and 5 f-RACE experiments, identified an ORF encoding a protein of 517 amino acids. The Pfam motif-finding software indicated that the putative Grv1 protein has a major facilitator superfamily (MFS) domain and phylogenic analysis of the domain revealed that the protein is a member of the anion:cation sympoter (ACS) family in MFS. The *grv1* gene is transcribed specifically in the stipe at early stages during fruiting- body maturation when rapid stipe elongation starts.

281. Sfp1p and rapamycin influence cell growth in a media-dependent manner in *C. albicans*.

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TOR (Target of Rapamycin) kinases are conserved nutrient-sensing regulators of cell growth in most organisms.Previous studies demonstrated that *C.albicans* is sensitive to rapamycin, and contains components of the TOR signaling pathway, including a single TOR kinase. However, the pathway has not been fully characterized. In order to understand how nutrient status influences the cell cycle and growth in *C. albicans*, we investigated the function of Sfp1p, a nutrient-sensing modulator of cell size, and known target of the TOR pathway in *S. cerevisiae*. Deletion of *SFP1* in *C. albicans* resulted in a decrease in cell size, but not to the same extent as that seen in *S .cerevisiae*. This effect was more evident in minimal vs rich media. Cells of the deletion strain also grew more slowly, forming smaller colonies than the control strain, and were more sensitive to rapamycin, suggesting that Sfp1p mediates a least part of Tor1p function in *C. albicans*. Transcription profiling of the deletion strain grown in different media is currently underway in order to identify potential targets of Sfp1p and tounderstand the mechanisms by which it influences cell size in a nutrient- dependent manner. Surprisingly, we also found that wild-type cells exposed to lethal concentrations of rapamycin did not die if the cells were grown on minimal vs rich media. This differential effect was not due to differences in pH of the media or the ability of the drug to enter the cells. Thus, TOR signaling involving Sfp1p is important for nutrient modulation of cell cycle progression and growth in *C.albicans*, and cells can differentially respond to an important anti-fungal drug depending on the environmental conditions.The mechanisms mediating this effect are currently being investigated.

282. Upregulation of cell cycle genes in *Epichloë festucae* during endophytic as compared to epiphytic growth

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Epichloë festucae is an endophyte of cool season grasses. It colonizes all above- ground plant organs, growing intercellularly as rarely branched hyphal strands. During inflorescence development the fungus exhibits a dual growth mode: on some tillers it infects inflorescences benignly and transmits clonally in seeds, on others it produces an ectophytic mycelium that becomes an ascogenous stroma, and chokes inflorescence development. Sequencing and annotation of the *E. festucae* genome allowed identification of mRNA models potentially involved in various cell cycle processes. Analysis of differential gene expression comparing stromata and benignly-infected inflorescences indicated that several of these genes are upregulated during endophytic, but not during ectophytic growth. Does the endophyte grow more prolifically during its hidden stages than during the easily discernible ectophytic growth stages? Could this indicate intercalary fungal growth, as proposed by Christensen et al. in 2008? Implications on fungal growth are discussed.

283. A split CandA regulates ubiquitin ligases in *Aspergillus nidulans*

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Cullins are a class of scaffold proteins, which are part of the SCF (Skp1/Cullin/F-box protein) ubiquitin ligases. The activity of these ligases is positively regulated by the neddylation of the cullin while binding of the cullin to Cand1 (cullin-associated Nedd8- dissociated protein 1) favors the disassembly and impairs the reassembly of the complex. Cand1 blocks the neddylation site in the C-terminal part of cullin as well as the Skp1 adaptor binding site in the N-terminal region of cullin. In *Aspergillus nidulans*, the gene encoding the putative homolog of Cand1 is split into two genes (*candA-N* and *candA-C*) encoding two proteins each with one cullin binding site. Deletion of either gene causes an impairment of the asexual cycle, a block of the sexual cycle and the production of brownish-red pigments. These defects can be complemented by a *candA-N::C* fusion construct. Both CandA proteins interact with each other, but only CandA-C associates with cullins in a yeast two-hybrid assay indicating that CandA-N binding to cullin is mediated by CandA-C. *In vivo*, CandA-N requires CandA-C for transport into the nucleus. Binding of only CandA-C to cullin might reflect transient states in the disassembly/reassembly cycle of SCF ubiquitin ligases which could be adopted independently of cullin deneddylation.

284. A new cell type produced by fungal spores that is involved in sexual reproduction

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The most common type of reproductive and dispersal structure in fungi is the asexual spore called a conidium. Conidia commonly produce two cell types, the germ tube, which is involved in colony establishment, and the conidial anastomosis tube (CAT), which is involved in forming hyphal networks during colony initiation. Here we describe in the model fungus, Neurospora crassa, a third cell type, the conidial sex tube (CST), which is involved in sexual reproduction. CSTs are long, thin, unbranched and septate with predominately uninucleate hyphal compartments, and are thus morphologically distinct from germ tubes and CATs. They also do not respond chemotropically to other CSTs, germ tubes or CATs but act as male fertilizing agents that attract female cells (trichogynes). Isolated conidia can be induced to form CSTs by synthetic sex pheromone of opposite mating type, a process that is pheromone receptor-mediated. Unlike germ tubes and CATs, CSTs seem to be covered in hydophobins which are required for CST formation. The CST represents an important new experimental system for analysing the regulation of cell differentiation and pheromone-mediated signal transduction in filamentous fungi.

285. The importance of e3 ubiquitin ligase scf complexes for the development of the mold *Aspergillus nidulans*

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Targetting to the proteasome often requires the phosphorylation of substrates and subsequent ubiquitination by a pathway involving the enzymes E1 activating, E2 conjugating and several types of E3 ligases. The largest class of E3 ligases is the cullin RING ligase (CRL), which the scaffold protein, CulA, is the target of Nedd8/RubA, an Ub like protein. We have previously shown that the deneddylation is necessary for fungal development (Busch et al., 2003; 2007). For further exploration of the role of RubA during fungal development, we have tagged RubA (TAP and/or S-tag) to identify the association partners of neddylated cullins. Neddylated Cul1/CulA recruits the counterparts of Skp1/SkpA, Rbx1/RbxA and various F-box proteins, which are core parts of CRLs. In addition, proteins required for the RubA linkage pathway were identified. A genetic analysis of the identified interacting proteins showed that the corresponding genes often are essential for *A. nidulans* growth. We showed this by heterokaryon rescue for the RubA encoding gene. Experiments with higher eukaryotes are difficult, because mutations often result in embryonic death of the organism. A deletion analysis revealed that not even parts of the *rubA* encoding gene can be deleted. CulA, RbxA, and RubA-E2 conjugating enzyme, UbcL, also seem to be essential. Busch et al., Mol. Microbiol. 49, 717-730, 2003. Busch et al., PNAS USA. 104, 8125-8130, 2007.

286. Regulation of cell elongation and branching is determined by differential phosphorylation state of COT1 kinase in *Neurospora crassa*.

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Disfunction of *cot-1* (encoding the founding member of the conserved NDR protein kinase family) confers pleotropic defects accompanied by restricted, colonial, growth. The significance of COT1 phosphorylation states on cell morphology *in vivo*, and on kinase activity (as measured *in vitro*) was analyzed in strains where conserved COT1 regulatory residues were substituted by either Glu or Ala (mimicking phosphorylated and unphosphorylated states, respectively). *cot-1*E198A *cot- 1*S417E, *cot-1*S417A, *cot-1*T589E and *cot-1*T589A were viable, yet impaired in hyphal elongation/branching. In *cot-1*S417A and *cot*-1T589E, elongation was affected to a greater extent than branching, suggesting that COT1 can regulate these processes in different manners. A 40% increase in the sensitivity of *cot-1*T589E to Caspofungin and Polyoxin D (inhibitors of glucan and chitin synthesis, respectively), supports a functional link between COT1 and cell wall integrity. Partial suppression of a *pod-6* ts strain (encoding a COT1-interacting STE20/germinal center kinase) by *cot-1*T589E, but not by *cot-1*S417E, indicates that POD6 plays a role in the phosphorylation of Thr589. COT1 kinase activity was significantly reduced in *cot-1*S417A, yet not in *cot-1*T589A, indicating that Thr589 phosphorylation is not essential for kinase activity.

287. The functional core of Cop9 signalosome plays the key role in regulating the activity of SCF E3 ubiquitin ligases.

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SCF-typed E3 ubiquitin ligases are one type of the best-understood multi-subunit complexes, which consist of the scaffold Cullin1, adapter protein Skp1 and substrate recruiting components F-box proteins. Cop9 signalosome (CSN) is a highly conserved complex, which is composed of eight subunits (CSN1-CSN8) in higher eukaryotes. Previous studies demonstrated that Cop9 signalosome (CSN) can cleave a ubiquitin-like protein, Nedd8, from Cullin1 to regulate the activity of SCF E3 ubiquitin ligases. Here we showed that in *Neurospora* CSN complex was made up of seven subunits. The individual subunit knockout mutants showed different phenotypes suggesting that they contribute differently to the function of Cop9 signalosome. Applying genetic and biochemical approaches, we demonstrated that the functional core subunits of CSN which were required for stability of SCF complex in *Neurospora*. These results suggest that in higher eukaryotes the functional core has the same roles in regulating the activity of SCF E3 ubiquitin ligases.

288. Natamycin specifically binds ergosterol, but does not form a membrane disrupting complex like other polyene antibiotics.

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Natamycin is used as an anti-fungal to prevent outgrowth of fungi on surfaces of cheese and sausages. It is a polyene antibiotic that binds specifically to ergosterol in model membranes and yeast cells. We have tested the effects of natamycin on germinating conidia of *Penicillium discolor*, a food-spoiling fungus and compared these with other polyenes namely, filipin and nystatin. Filipin is fluorescent and strongly stains ergosterol is strongly stained at the site of germ-tube emergence after isotropic growth. The fluorescent dye TOTO-1 shows that the integrity of the plasma membrane is maintained in the presence of natamycin. In contrast, the polyenes filipin and nystatin caused dose- and time dependent influx of these fluorescent compounds. ESR-studies confirmed the integrity of the conidial membrane in the presence of natamycin, but showed differences between filipin and nystatin. These results suggests that natamycin unlike other well-known polyenes does not form membrane disrupting complexes, but interferes with ergosterol functioning in the cell. For example, experiments with the endocytic marker FM4-64 suggest that natamycin can be used as a specific inhibitor for early endocytosis.

289. A molecular dissection of the Cryptococcus unisexual reproduction cascade

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The basidiomycete *Cryptococcus neoformans* is a common global human pathogen. Human infection is thought to be caused by inhalation of spores from the environment. The small diameter of spores facilitates alveolar penetration, and following germination the yeast disseminates hematogenously. In the laboratory, nutrient limitation and pheromones induce a dimorphic transition from yeast to hyphae via sexual reproduction. The traditional sexual cycle involves α and a opposite mating type cells. Monokaryotic fruiting is a modified sexual cycle involving cells of the same mating type, usually α. Both forms of sexual reproduction lead to the formation of hyphae and basidia, where meiosis followed by multiple rounds of mitosis produces infectious basidiospores. The conserved MAP kinase and cAMP-protein kinase signaling cascades control mating; however, the pathway targets are largely unknown. Here we applied insertional mutagenesis to identify genes governing unisexual reproduction. A hyper- filamentous strain was subjected to T-DNA mutagenesis using *Agrobacterium tumefaciens*. 6,100 mutant strains were generated, of which 225 exhibited filamentation defects on V8 media. These mutants were categorized based on phenotype: afilamentous (20), short filaments (111), hyperfilamentous (73), increased chlamydospore production (5) and dense, sparse filaments (9). Four mutants altered in pH or light responses were also obtained. Representative mutants from each group have been selected for further characterization. Our aim is to identify and characterize novel unisexual mating pathway components in *Cryptococcus*.

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290. The exocyst in *Neurospora crassa*: a tale of vesicles, fusions and apical growth.

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The last stage of the secretory pathway from the *trans*-Golgi to the plasma membrane (PM) is exocytosis, a process requiring high fidelity protein-protein interactions to fuse secretory vesicles to the PM. The exocyst is a conserved octameric complex consisting of SEC-3, -5, -6, -8, -10, -15, EXO-70 and -84. It marks the site of vesicle fusion to the PM, and is regulated by several GTPases (SEC-4, RHO-1, RHO-3, CDC-42). *Neurospora crassa* has single genes for all eight exocyst components. We constructed translational fusions of all exocyst genes with GFP, 3xHA and/or FLAG tags at their endogenous loci by a split marker "knock-in" procedure. The localization and dynamics of the GFP fusions was examined in living hyphae by laser scanning confocal microscopy. For all tagged exocyst components, GFP fluorescence accumulated primarily adjacent to the plasma membrane at the hyphal dome, which correlates with the place of intensive exocytosis in polarized growth. A low GFP fluorescence was observed around the pore of newly formed septa, while a strong fluorescence was noticeable at sites of hyphal contact in anastomosed hyphae and in older septa. By crosses and heterokaryon formation, we generated strains that carry exocyst genes with different tags and verified the expression of all fusion proteins by western blot. We are co-immunoprecipitating exocyst components to uncover exocyst assembly pathways and important interaction domains. Our cytological data suggest that the exocyst is involved in vesicle fusion during apical growth and during pore development both in septa and in fusing hyphae.

291. Expression of the G-protein coupled receptor Bar2 in the basidiomycete *Schizophyllum commune*.

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The sexual development of the heterothallic basidiomycete *Schizophyllum commune* depends on a tetrapolar mating system. The mating type specific pheromone receptor Bar2 belongs to the G-protein coupled, seven transmembrane domain receptors. The importance of the pheromone/receptor system is well- investigated, however there is little information available on the expression and the localization of the pheromone receptor. By means of real-time PCR, the expression level of receptor and pheromone genes was determined in mated mycelia over a 72-hour time period. In compatible wildtype strains very low expression levels which increased transiently during mating interactions were revealed. The same pattern but lower expression levels were seen when a receptor transformant was used in mating interactions. C-terminal truncated receptor transformants are notable because of the occurrence of unfused clamp cells in a compatible mating interaction. As the intracellular C-termini of pheromone receptors in *S. commune* are long compared to other fungal species, an additional function may be proposed to be located there. *S. commune* strains expressing a Gfp-fused receptor were investigated by confocal laser scanning microscopy. Expression of the Gfp fusion protein was seen in unfused clamp cells which could be well visualized in transformants containing the truncated receptor due to their phenotype of retarded clamp fusion.

292. Spatial stratification of Beta-1,3-glucan- and chitin-synthesizing enzymes in the spitzenkörper of *Neurospora crassa*.

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Chitin and beta-1,3-glucan synthases play a crucial role during the morphogenesis of filamentous fungi. Previous studies have shown that chitin synthases (CHS) are delivered to the actively growing hyphal tip by secretory microvesicles that follow an unconventional route. In this study, we chose to examine the subcellular localization of GS-1 (ncu04189) from *Neurospora crassa* as a reporter of the beta-1,3-glucan synthase complex. GS-1 is a protein essential for beta-1,3-glucan synthesis and, as we show here, associated to high-density particles that cosediment with beta-1,3-glucan synthase activity, an indication of their close relationship in a catalytic complex. Upon molecular fusion with two fluorescent proteins GS-1 was observed in a dynamic and pleomorphic apical structure, usually arranged as a ring, which corresponds to the outer stratum of the Spitzenkörper (Spk). GS-1 reaches the hyphal apex conveyed by heterogeneous-size particles that move along defined paths presumably microtubules, as suggested by TIRF microscopy and impaired GS-1 secretion after benomyl treatment. On sucrose density gradients, the GS-1-associated particles showed a buoyant density distinctly higher (1.138-1.198 g/ml) than that of CHS-carrying chitosomes (1.127 g/ml). Confocal examination of growing hyphae of *N. crassa* dually labeled with GS-1- GFP and CHS-1-mCherryFP showed clearly the stratification of these proteins in the Spk. These findings, taken together with the previously reported subcellular localization and secretion pattern of CHS, reveal that the components of the cell wall building apparatus of *N. crassa* are delivered to the apex in at least two different types of secretory vesicles that accumulate in different strata of the Spk, a differentiation presumably related to the spatial control of cell-wall synthesis and hence morphogenesis.

293. Strain improvement of Trichoderma using green fluorescent protein and fluorescence activated cell sorting

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The filamentous fungus *Trichoderma reesei* is an important source of hydrolytic enzymes in nature and industry. Germlings of *Trichoderma reesei* were used for directed evolution to improve secretion of enzymes for industrial production using high speed fluorescence activated cell sorting (FACS). To determine if flow cytometric measurements of protein expression could be made on germlings we created a gene construct placing the *Renilla reniformis* green fluorescent protein (GFP) gene under control of the cellobiohydrolase I (cbh1) promoter and terminator of T. reesei. This vector was transformed into the genome of T. reesei and GFP expression was measured in germlings by flow cytometry. Green fluorescence was observed in germlings grown under conditions known to produce cellulase expression in *Trichoderma*. Spores were mutated with UV light and growing germlings were screened using FACS, yielding improved mutants.

294. Understanding chitosan mode of action in filamentous fungi

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Chitosan, inhibits fungal growth and spore germination in plant pathogenic fungi, but there is lack of knowledge about its effect in biocontrol fungi. We have tested chitosan effect on germination and growth of fungal root pathogens and biocontrol fungi (nematophagous, entomopathogenic and mycoparasites). We observed large differences in the effect of chitosan in both groups of fungi. Chitosan inhibied spore germination and reduced growth of plant pathogenic fungi but had a low inhibition in nematophagous and entomopathogenic fungi. This could at least partly explained by the presence of chitosan degrading enzymes in biocontrol fungi. Plasma membrane damage has previously been suggested to explain the toxic effects of chitosan on filamentous fungi. To study fungal cell death associated with plasma membrane permeabilization by chitosan we used *Neurospora crassa* as experimental system. Rhodamine-labelled chitosan was used to show chitosan internalization by fungal cells. Cell viability stains and the calcium reporter, aequorin, were used to monitor plasma membrane permeabilization and cell death. Chitosan permeabilization of the fungal plasma membrane and its uptake into fungal cells was found to be energy dependent.

295. The iron-regulated gene CIG1 of *Cryptococcus neoformans* is required for cell wall integrity, iron sequestration and secretion.

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Cryptococcus neoformans is a fungal pathogen that causes cryptococcosis, the most common form of fungal meningitis worldwide. In *C. neoformans*, iron is a key nutritional cue necessary for regulating expression of virulence factors. A predicted cytokine inducing glycoprotein (CIG) was previously found to be the most abundantly expressed gene in *C. neoformans* when grown in low iron media and was differentially expressed when grown in media with or without iron. We demonstrated that the Cig1 protein is localized non-uniformly at the cell surface (cell wall, capsule) and associated with extracellular vesicles secreted by *C. neoformans*. These extracellular vesicles are thought to transport the polysaccharides needed for capsule formation, suggesting a possible link between Cig1 and the capsule formation. Additionally, a CIG1 disruption mutant did not secrete as much laccase or phosphatase as the wild type strain and showed a growth defect when grown in presence of secretion inhibitors, indicating a role for Cig1 in protein secretion. The CIG1 mutant had a cell wall defect and a marked growth defect at 37ºC and in presence of different agents that challenge the cell wall integrity, suggesting a role for Cig1 in cell wall integrity. We have also shown that Cig1 is involved in iron sequestration at the cell surface as the CIG1 mutant did not sequester as much radioactive iron as the wild type strain. Thus the Cig1 protein is an iron-regulated protein that is involved in secretion, cell wall integrity and iron sequestration.

296. Exploring fungal biology using light-, electron- and spectro-microscopy.

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For over a century, studies of the growth of mycelia and of individual hyphae in culture using microscopy, has generated and reinforced the paradigm that most of the really important events are at extending tips. In part, this has been due to the disparity in spatial resolution between microscopy and chemical analyses. Chemical analyses had been limited to a few targets at high spatial resolution (e.g. immuno-based and chemical- specific probes, monitored using microscopy) or high chemical resolution of bulk samples (e.g. mass spectroscopy). However, fungal hyphae change in structure and function within nanometers to microns, and vary between individual hyphae in a common environment. Ongoing development of high spatial resolution spectromicroscopic methods has bridged this gap. Here, we show preliminary correlative studies using light and electron microscopy and high spatial resolution chemical analyses: Fourier transform infrared spectroscopy and X-ray fluorescence spectroscopy (both using brilliant synchrotron light sources); Raman and SERS spectroscopy; imaging mass spec using laser micro-ablation. Together, we are using these techniques to better understand the roles of tip and basal hyphal regions in saprotrophs, pathogens, and endophytes. Using complementary methods has allowed us to begin to refine our ideas of how fungi interact with their physical and biological environments. Supported by NSERC (KG and SK), CIHR (KG), CIHR-RPP (SK)

297. Dynamics of nuclear localization *in vivo* of the *frq* gene product in *Neurospora crassa* during the circadian rhythm

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The circadian rhythm of *Neurospora crassa* is expressed on the surface of an agar medium as alternating bands of conidiating regions from actively growing cultures. Almost all of the molecular information about this rhythm has been obtained in underwater disk shaking cultures that are not actively growing. To reconcile this two drastically different culture conditions, a new set of tools have been developed, ie the use of a red fluorescent protein (RFP) as a reporter in combination with time-lapse confocal microscopy. This reporter was codon optimized for expression in *Neurospora* and was named mCherryNC. The expression of mCherryNC driven by the *ccg-2* (clock- controlled gene) promoter was examined in vegetative hyphae of growing cultures under fluorescence and confocal microscopy. RFP showed temporal expression, reflecting the circadian control of the *ccg-2* gene and spatial expression, ie it was localized to aerial hyphae and conidia. The mCherry reporter was also fused to the C-terminal end of the FRQ (frequency) protein and examined during a complete circadian rhythm under the control of the *ccg-2* and *frq* promoters. The fluorescence of FRQ-mCherry, driven by either promoter, was detected only at the growing edge of the colony; it was observed both in the cytoplasm and nuclei of vegetative hyphae for a distance of approximately 150-200 μm from the cell apex. Accumulation of FRQ-mCh in nuclei under the control of the *ccg-2* or *frq* promoters showed two peaks of fluorescence. Clearly, the RFP mCherryNC proved to be a new and useful tool to monitor the circadian rhythm at the cellular level in growing cultures of *N. crassa*.

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298. Timely septation requires SNAD-dependent spindle pole body localization of the septation initiation network components in the filamentous fungus *Aspergillus nidulans*.

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In the filamentous fungus *Aspergillus nidulans*, cytokinesis/septation is triggered by the kinase cascade of the septation initiation network (SIN) which first appears at the spindle pole body (SPB) during mitosis. The novel coiled-coil protein SNAD is associated with the SPB, and is required for timely septation, and conidiation. We have determined that SNAD acts as a scaffold protein that is required for the localization of the SIN proteins of SIDB and MOBA to the SPB. Another scaffold protein SEPK/SNAE, whose localization at SPB was dependent on SNAD, was also required for SIDB and MOBA localization to the SPB. In the absence of either SEPK/SNAE or SNAD, SIDB/MOBA successfully localized to the septation site, indicating that their earlier localization at SPB was not essential for their later appearance at the division site. Our results suggested that through SEPK/SNAE, SNAD mediates the interaction between SIN components and cell cycle regulators at the SPB. Unlike their functional counterparts in fission yeast, SEPK/SNAE and SNAD were not required for vegetative growth except for timely septation. Furthermore, hyperactivation of the SIN pathway by downregulation of negative regulators of the SIN suppressed the phenotype of aborted conidiation due to the loss of SNAD. Therefore, we conclude that SPB localization of SIN components is not essential for septation *per se*, but critical for septation to take place in a timely fashion. In addition, we conclude that timely execution of septation is a prerequisite for conidiation.

299. Carbon-ion beam sensitivity of DSB repair-deficient mutants of *N. crassa*

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Carbon-ion beam is known to affect a cellular components of the living organism to make a severe injury. Its irradiation to cells often causes the DNA double strand breaks (DSBs). DSBs are mainly repaired via either of two DSB repair systems; non- homologous end-joining (NHEJ) or homologous recombination (HR). Further, these systems are controled by MRX (Mre11-Rad50-Xrs2) complex. The filamentous fungus *N. crassa* also has almost all of the members. Our interest is how HR, NHEJ and MRX correlate to repair the DNA damages arising from carbon-ion irradiation in *N.crassa*. Carbon-ion beam were irradiated to wild type and three DSB repair- deficient mutants such as *mus-52* (YKU80), *mei-3* (RAD51) and *uvs- 6* (RAD50). The *uvs-6* mutant showed the highest sensitivity to the carbon-ion beam among the tested strains. Sensitivity of the *mus-52* mutant is higher than that of the wild type and *mei-3* when the dose is under 50 Gy, and lower than that of *mei-3* and *uvs-6* when it is over 100 Gy, and lower than wild type when it is over 200 Gy. Therefore, damages induced by higher doses (>100 Gy) will be mainly repaired by HR, but in lower doses (<50 Gy) those may be mainly repaired by NHEJ. The frequency of induced mutations in the *ad-3* (*adenine-3*) loci was determined by measuring the number of forward mutations. Mutation frequency of the *mus-52* strain decreased approximately 1.8-fold compared to wild type. Mutation frequency of the *mei-3* strain increased approximately 3-fold compared to wild type. The mutation spectrum in *ad-3A* loci was determined.

300. The H^+-ATPase is localized in the plasma membrane in subapical hyphal regions in *Neurospora crassa*.

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In filamentous fungi, a highly polarized traffic of secretory vesicles and their cargo delivers newly synthesized membrane proteins to the hyphal apex. Before tethering, docking and fusion of secretory vesicles to the plasma membrane (exocytosis) they aggregate temporarily at the Spitzenkörper. One of the unanswered questions about the secretory pathway in fungal hyphae is whether all secretory vesicles accumulate at the Spitzenkörper. As part of an ongoing project to characterize the organization of the secretory pathway in filamentous fungi, we have set out to localize the plasma membrane H^+-translocating ATPase along hyphae of *Neurospora crassa*. The H^+- ATPase protein PMA-1, encoded by *pma-1*, is carried by secretory vesicles from their point of synthesis in the ER to their destination. PMA-1 is delivered via the secretory pathway to the cell surface, where it pumps H^+ out of the cell, generates a large electrochemical gradient and supplies energy to H^+-coupled nutrient uptake systems. We have fused the *pma-1* gene from *N. crassa* to *gfp* and studied its expression in living hyphae of *N. crassa* FGSC # 9717. Transformants showing positive fluorescence were selected and analyzed by confocal laser scanning microscopy. The GFP labelled H^+-ATPase did not accumulate at the Spitzenkörper. PMA-1-GFP was found at septa and in the cell surface of subapical regions (~50 μm). Our results confirm earlier predictions that the H^+-ATPase is deficient or inactive at the apex but abundant distally, and provide insight into one of the different pathways for the delivery of proteins from Golgi to the cell surface.

301. Molecular analysis of Ras-specific signaling pathways in *Ustilago maydis*

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Ras-GTPases act as molecular switches and exist in the active GTP-bound state and the inactive GDP-bound state. In their GTP-bound form they activate effector proteins that are necessary for the regulation of many cellular processes. *Ustilago maydis*, a dimorphic phytopathogenic fungus, is an excellent model organism to study the role of Ras-GTPases. There are two Ras proteins expressed in *U. maydis*, Ras1 and Ras2. Ras2 was previously shown to be involved in MAPK signaling during pathogenic development. We could demonstrate that Ras1 is an essential protein that regulates cell polarity, nuclear distribution and vacuolar morphology. Our research addresses upstream activation of Ras-GTPases as well as the downstream effectors. The genome of *U. maydis* comprises five putative guanine nucleotide exchange factors (GEFs) catalyzing the GDP/GTP exchange of Ras proteins. We determined the specificity of these Ras-GEFs towards Ras1 and Ras2 using *in vitro* GEF assays. For the study of downstream effectors we have analyzed five Ras association (RA) domain containing proteins in *U. maydis*. We could show that Ras1 is able to activate the MAPK-cascade involved in pathogenic development and the cAMP pathway. The RA-domain containing protein Bud14 participates in the regulation of cell morphology. We have evidence that Ras1 activates the Rho-GTPase Rac1 and we assume that this activation is mediated via the Rac1-specific GEF Cdc24.

302. RdsB governs iron acquisition and virulence in *Aspergillus fumigatus*

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To identify transcriptional regulators of virulence in *A. fumigatus* (AF) we studied RdsB, which is a Zn(II)Cys(6) transcription factor that was identified by its homology to Cwt1 in *Candida albicans* (CA) and Rds2 in *Saccharomyces cerevisiae* (SC). In AF, *RdsB* expression was highest in swollen conidia and declined progressively during hyphal extension and maturation. Although Cwt1 and Rds2 govern cell wall structure in CA and SC, the AF *Delta-rdsB* mutant had normal susceptibility to the cell wall stressors, Congo red and calcofluor white. In vitro, the *Delta-rdsB* mutant was slightly more susceptible than wild-type (WT) and *Delta-rdsB::rdsB* complemented (COMP) strains to killing by neutrophil-like or macrophage-like cell lines. However, it was similar to the WT strain in its capacity to adhere to and invade endothelial cells and the A549 pulmonary epithelial cell line. The *Delta-rdsB* mutant had significantly reduced virulence when injected into *Galleria mellonella* larvae. It also had reduced virulence in non-neutropenic mice that were immunosuppressed with cortisone acetate, as determined by competition experiments and survival studies. Microarray analysis of the *Delta-rdsB* mutant grown in RPMI 1640 medium revealed that RdsB governs the expression of 10 genes whose products are involved in high affinity iron uptake. These genes encoded siderophore biosynthesis enzymes, siderochrome-iron transporters, and iron permeases and reductases. As predicted by these results, the *Delta-rdsB* mutant grew more slowly than the WT and COMP strains under iron-limited, but not iron- sufficient conditions. Therefore, RdsB helps regulate iron acquisition under low iron conditions, and is required for maximal virulence of AF. [*]Presenting author (E-mail: hliu@labiomed.org)

303. Fruiting body development in *Coprinopsis cinerea*

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Fruiting body development in the higher basidiomycetes is the most complex process occurring in the fungal kingdom. Fruiting body development in the model species *Coprinopsis cinerea* is well adapted to the normal day-night rhythm and it requires both dark and light phase to go through the whole developmental pathway. In total, the process takes 7 days during which a succession of defined events happens. Under normal circumstances, fruiting requires 25°C and light is absolutely essential for induction. The *A* and *B* mating type genes control initiation and light regulation links to the pathway of mating type control. We present conditions under which the tight control by temperature and light is overcome. Fruiting initiates at 37°C in the dark after by addition of high levels of copper ions to the medium. It is possible that as recently shown in *Cryptococcus* by Kent et al. (AEM 74:6248) copper up-regulates the mating type genes.

304. Functional analysis of three Ca^{2+}-calmodulin-dependent kinases in the endophytic fungus *Epichloë festucae*.

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Ca^{2+}/calmodulin-dependent kinases are involved in signaling pathways responsible for the regulation of various processes in filamentous fungi, including cell cycle, nuclear division, circadian clock, hyphal growth, sporulation and pathogenicity. *E. festucae* is an endophytic filamentous fungus that forms a mutualistic symbiotic association with perennial ryegrass. Recent research has shown that fungal signaling pathways play a crucial role in the regulation of this symbiotic interaction. The aim of this research is to characterize three genes encoding Ca^{2+}/calmodulin- dependent kinases, camkA, camkB and camkC, and investigate their role in fungal growth and symbiosis. Single knock-out strains of all three genes have been generated, showing that these genes are not essential for growth of *E. festucae*. The growth rate, colony morphology, hyphal morphology and conidiogenesis of the deletion strains were the same as wild type. Moreover, knock-out strains do not appear to have increased sensitivity to high salt concentrations or cell wall destabilizing agents, compared to wild type. Analysis of the symbiotic phenotype for each deletion strain is currently underway.

305. An *Agrobacterium tumefaciens* mediated T-DNA insertion in *Epichloë festucae* disrupts the mutualistic interaction of this endophyte with its host perennial ryegrass.

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The mutualistic symbiosis between the fungal endophyte *Epichloë festucae* and its host, *Lolium perenne*, is an ideal experimental system to study the signalling required for symbiosis maintenance. In wild-type symbiota, hyphal growth is tightly synchronized with growth of the leaves. Mutants of *E. festucae* that disrupt this pattern of growth give rise to a stunted host phenotype. The aim of this study is to identify additional fungal genes required for symbiosis maintenance by screening T-DNA insertional mutants that cause a stunted host phenotype. In contrast to wild-type, growth of mutant TM1066 in leaves was unregulated with multiple hyphae in the intercellular spaces as well as the vascular bundles, a tissue rarely colonized by the wild type. However, epiphyllous growth of this mutant was dramatically reduced. Tail PCR was used to rescue sequences flanking the LB junction of this single copy T- DNA insertion mutant. Sequence analysis revealed homology to a pseudouridine synthetase. Further molecular analysis identified a 1.1-kb deletion associated with the T- DNA insertion, resulting in truncation of the 3' region of the pseudouridine synthetase as well as the 3' region of an adjacent MAPKK, homologous to the *S. cerevisiae* Mkk2, a component of the cell wall integrity/cell cycle MAPK pathway. We are currently testing the hypothesis that deletion of the MAPKK was responsible for the symbiotic phenotype by analyzing a targeted disruption of the *mkk2* and by testing whether the wild-type gene will complement the original insertion/deletion mutation associated with TM1066.

306. Dynamic nuclear transport within Neurospora colonies.

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Time lapse confocal microscopy was used to image movement of GFP tagged nuclei within living colonies of *Neurospora*. In addition, vital dyes were used to show localisation of organelles/membranes. Movement of nuclei was observed over several millimetres and the direction was generally towards the leading edge of the colony. Time lapse movies illustrate the unique dynamics of nuclear movement within living hyphae. The data suggests that long distance genetic transport occurs within fungal colonies.

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307. "Give me Pdc1 and Uro1 or give me death": Two proteins required for viability of *Ustilago maydis*.

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14-3-3s and Rho-GTPases are two distinct families of functionally-conserved eukaryotic proteins that participate in many important cellular processes such as signal transduction, cell cycle regulation, stress response, and apoptosis. However, the exact role(s) of these protein families in these processes is not entirely understood. We were able to demonstrate a connection between the homologues of 14-3-3epsilon and Rho1 for the maize pathogen, *U. maydis*. Initial experiments suggest that Pdc1, a homologue of 14-3-3epsilon, regulates cytokinesis, chromosomal condensation, and vacuolar formation. Similarly, Uro1, a homologue of Rho1, is also involved in these three essential processes, plus mating and filamentation. Extended depletion of either Pdc1 or Uro1 levels is lethal for cells. Yeast-2-hybrid and epistasis experiments suggest that both Pdc1 and Uro1 participate in the same regulatory cascade(s) controlling cell growth and filamentation in *U. maydis*. Over-expression of Uro1 ameliorated the defects of Pdc1-depeletion. Interestingly, the lethality due to extended knock-down of either gene is completely suppressed in the strain deleted for another small G protein, Rac1. Furthermore, deletion of *cla4*, encoding the effector kinase for Rac1, also rescued cells depleted for Pdc1. These data imply that Uro1 and Pdc1, via Uro1, are negative regulators of Rac1 and Cla4. We present a model for Pdc1 and Uro1 functions based on these and published data.

308. How do you PAK a fungus into a grass?

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In the mutualistic association between the fungal endophyte *Epichloë festucae* and perennial ryegrass, fungal growth is highly regulated and co-ordinated with host growth. Reactive oxygen species (ROS) produced by the NADPH oxidase (Nox) complex are integral to this signaling. Mutants in components NoxA, NoxR or RacA display deregulated growth *in planta*, and induce host stunting and senescence. In mammalian systems Nox activation requires translocation of NoxR and RacA to the plasma membrane. Under non-activated conditions RacA is bound by a RhoGDI, and its release is triggered by a p21-activated kinase (PAK). By analogy we propose that in *E. festucae* a PAK activates Nox via RacA release. To investigate this, the two *E. festucae* Pak genes were disrupted. Disruption of *pakA* resulted in altered branching, reduced conidiation and altered compartment size in culture. Staining for superoxide and hydrogen peroxide also revealed increased levels of both these species in culture, however, they were still highly localized to the hyphal tip. *In planta* the *pakA* mutant displayed unregulated growth and induced severe host stunting and premature senescence within 4 weeks after planting, likely due to extensive colonization of the host vascular tissue. Disruption of *pakB* resulted in a milder phenotype both in culture and *in planta*. In culture the mutant displays altered branching and hyperconidiation. This is unexpected as other fungal mutants in this gene generally show reduced conidiation. Whilst growth of this mutant *in planta* is less regulated than wild-type, the host plant remains asymptomatic.

309. Characterization of candidate Cdc42/Rac1 GEFs and GAPs in *Aspergillus nidulans*.

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The related GTPases Cdc42 and Rac1 act via multiple effectors to regulate cellular morphogenesis in eukaryotes. We have previously shown that Cdc42 and Rac1 share at least one essential function required for polarized hyphal growth in *Aspergillus nidulans*. In addition, they appear to possess distinct functions, such as in the regulation of NADPH oxidase activity and the control of hyphal branching. To further understand the roles of Cdc42 and Rac1 in hyphal morphogenesis, we have undertaken the characterization of their respective GEFs (guanine nucleotide exchange factors) and GAPs (GTPase activating proteins). We have identified three candidate GEFs that may promote activation of Cdc42 and/or Rac1. Our functional studies show that one of these, Cdc24, is essential and likely activates both GTPases. Loss of Cdc24 leads to the formation of swollen spores incapable of establishing a stable polarity axis. By contrast, FubA (= mammalian tuba) and DckA (= mammalian DOCK180) appear to have subtler roles in morphogenesis and development that may reflect their specificity towards Cdc42 and Rac1, respectively. We have also identified a candidate GAP, RgaA, that likely down- regulates both Cdc42 and Rac1. Loss of RgaA triggers morphological phenotypes that are seemingly caused by hyper-activation of both Cdc42 and RacA, including expanded growth zones at hyphal tips and loss of apical dominance. Our results suggest that a suite of GEFs and GAPs confer precise spatial and temporal regulation of the activity of Cdc42 and Rac1 during hyphal morphogenesis and development.

310. The grass endosymbiont, *Epichloë festucae*, forms epiphyllous mycelial nets on leaves of *Lolium perenne*

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Fungal endophytes of the genera *Epichloë* /*Neotyphodium* are well known biotrophic fungi that colonise the intercellular spaces of aerial tissues of Pooid grass species. However, some of these fungal species have also been shown to establish epiphyllous mycelial nets on the leaves of their host grass (Moy et al. 2000). The aim of this study was to examine whether *E. festucae* in association with *Lolium perenne*, establishes epiphyllous nets and whether mutations in genes previously shown to be essential for symbiosis maintenance, including *noxA* and *noxR*, affect epiphyllous growth. SEM analysis of young leaves of *L. perenne* infected with *E. festucae* revealed the presence of mycelial nets on the adaxial surface of the leaves. SEM and TEM analysis suggests that these hyphae emerge from the leaves by formation of internal appressoria-like structures. An unusual feature of these nets is the formation of coiled structures that were also found in axenic cultures of *E. festucae*. We propose that these structures promote localised hyphal branching and expansion of the net. Plants infected with the *noxA* and *noxR* mutants showed reduced epiphyllous growth compared to wild-type. However, these mutants did form an extensive hyphal network below the cuticle, suggesting they were unable to breach this surface barrier. As proposed by Moy et al. (2000) epiphyllous mycelial nets may play a role in defense of host plants from potential pathogens through 'niche exclusion'. Moy et al. (2000). Symbiosis 28: 291-302.

311. BEM-1 is required for directed but not for general polar growth in *Neurospora crassa*
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The molecular mechanisms mediating cell cell fusion are only poorly understood. We are using *Neurospora crassa* as a model system to study cell fusion and related signaling mechanisms. Germinating *N. crassa* spores show chemotropic growth towards each other and fuse. As a result individual germlings become one functional unit, which subsequently develops into the mycelial colony. Earlier studies have shown that the MAP kinase MAK-2 is an essential part of a signaling cascade involved in germling fusion (Pandey et al. 2004). To further characterize this signaling pathway we analyzed the role of BEM-1, a possible scaffold for MAK-2. The orthologous protein Bem1p in *Saccharomyces cerevisiae* is involved in polarity establishment during budding and shmoo formation. In contrast, a *N. crassa bem*-1 knock out mutant is not significantly impaired in spore germination and polar hyphal growth. However, *bem*-1 germlings are fusion defective and exhibit no chemotropic interaction. We determined the subcellular localization of BEM-1 by using GFP fusion constructs. We detected BEM-1-GFP accumulation at growing hyphal tips similar to previously reported data from *Aspergillus nidulans* (Leeder and Turner, 2008). In addition we found two more regions of BEM-1-GFP accumulation. In germling fusion pairs, BEM-1-GFP localizes to the tips of both partners and concentrates at the fusion point, once cell-cell contact has been established. Deconvolution microscopy and subsequent 3-D reconstruction revealed BEM-1-GFP localization around the opening fusion pore, detectable as a bright ring. We also detected BEM-1-GFP at septa of germ tubes and mature hyphae. Taken together, our data suggest novel functions of BEM-1 in chemotropic growth, fusion pore formation and septa formation or maintenance. In our further studies we will try to unravel and identify the distinct molecular functions of BEM-1 during *Neurospora* development.

312. Functional analysis of the conserved BEM46-like protein from *Neurospora crassa*
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We present data regarding the expression and function of the Neurospora gene *bem46*. The deduced amino acid sequence of this gene appears to be conserved among eukaryotes. We identified a homologous sequence some years ago in the filamentous fungus *Ascobolus immersus*, which was located near a truncated transposable element. When the Neurospora crassa genome sequence became available, we continued working with the *bem46* gene of *N. crassa*. Mutations were introduced using the RIP technique. However, ascospores of strong RIP mutants of *bem46* do not germinate or terminate germination at an early stage. We therefore established strains over expressing *bem46*, a *bem46:egfp* fusion, and a RNAi construct down regulating bem46 expression. Strains over expressing *bem46* and a RNAi construct exhibited abnormal ascospore germination, i.e. early growth arrest of germ tubes. Vegetative hyphae, perithecia, and ascospores develop normally. The BEM46 protein is targeted to the ER, and also localizes at or close to the plasma membrane in distinct areas. Our data suggest BEM46 may play a role in a signal transduction. This finding also implies a higher degree of differentiation of fungal hyphae than currently expected. We currently work on over-expression of BEM46 in *E. coli* to generate antibodies and also employ the yeast two-hybrid system to identify interacting proteins.

313. The roles of the polo like kinase PLKA in cell cycle regulation and development in *Aspergillus nidulans*.
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The Polo-Like Kinases (PLK) play critical roles throughout the cell cycle, and are essential in organisms that contain a single homologue. We previously identified PLKA, a polo-like kinase in *Aspergillus nidulans* that is the largest member of the PLK family to date. To directly investigate PLKA function, we recently created a conditional strain carrying a single copy of *plkA* under control of the *alcA promoter*, as well as a deletion strain. Surprisingly, our results show that *plkA* is not essential, despite being the single PLK in *A. nidulans*. However, it is required for proper colony growth and polar axis formation in hyphae, since strains lacking PLKA grew as compact colonies and contained multi-branched hyphae with split tips. Numerous abnormal spindle patterns were also present, and a significant proportion of cells contained telophase spindles, suggesting roles for PLKA in spindle formation and possibly mitotic exit. Chromosome segregation was also abnormal in a significant proportion of cells. Deletion of *plkA* did not affect nuclear division in hyphae, but partially suppressed the G2/M block in $nimT^{Cdc25C}$ cells at restrictive temperature, suggesting a novel role for PLKA in negatively regulating the G2/M transition. Surprisingly, PLKA was not required for septation, unlike that seen with other PLK. PLKA could be functioning in part through regulating microtubule dynamics, since the compact colony growth phenotype was cold-sensitive, and suppressed by low doses benomyl. Finally, cells lacking PLKA showed a decrease in the production of asexual structures but induction of sexual cell types including Hulle cells, suggesting a potential role for PLKA in regulating developmental processes. Thus, PLKA appears to have conserved and novel functions in cell cycle regulation in *A. nidulans*, and may contribute to a link between the cell cycle and development.

314. Cdc24-induced Rac1 GDP/GTP cycling is required for establishing cell polarity in *Ustilago maydis*
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Rho-type GTPases are involved in the regulation of many intracellular processes, such as organisation of the cytoskeleton, cell polarity and control of gene expression. GTPases cycle between two conformations, the active GTP-bound form and the inactive GDP- bound form. The temporal and spatial activation of GTPases depends on their specific activation by guanine nucleotide exchange factors (GEFs). In the dimorphic fungus *Ustilago maydis* homologs of the highly conserved GTPases Rac1 and Cdc42 have been identified. Both proteins are very similar in their amino acid sequence but function in different signalling pathways. Interestingly, Rac1 and Cdc42 share many downstream targets, among them the PAK-like kinase Cla4. Therefore we asked whether selective activation of Rac1 and Cdc42 by their cognate GEFs could determine signalling specificity. GEF assays demonstrated that Cdc24 acts specifically on Rac1 but not on Cdc42. Using biochemical and genetic analysis we could show that Cdc24 forms a signalling module that includes Rac1, Cla4 and the scaffold protein Bem1. Formation of this complex resulted in negative feedback regulation of Cdc24 at the protein level. We used this Rac1-dependent degradation of Cdc24 as readout to determine the activity of the Rac1/Cla4/Bem1 signalling module *in vivo*. We present a model for polar growth in which specific activation by the GEF Cdc24 determines Rac1 signalling specificity.

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315. Dissecting the Ras1 signaling cascade in *Cryptococcus neoformans.*

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Cryptococcus neoformans is an opportunistic human fungal pathogen that is the causative agent of cryptococcosis, a life-threatening infection. The ability to grow at high temperature is an important requirement of *C. neoformans* to establish disease. Previously we found that the *C. neoformans* Ras1 GTPase functions in a signaling cascade consisting of Cdc24 and Rho-like Cdc42 homologs to mediate cell polarity in response to mild stress, including growth at high temperature. In addition, Ras1 is also required for cell fusion, an early step of the *C. neoformans* sexual differentiation process. This process is independent of Cdc24 and the Cdc42 homologs. We demonstrate that localization of Ras1 to specific membranes in the cell is a mechanism of signaling specificity that promotes the interaction of Ras1 with specific effector proteins to mediate stress response and sexual differentiation.

316. Eln1, a yeast Cdc3-like septin in *Coprinopsis cinerea*, is involved in both stipe cell elongation and tip growth of vegetative hyphae

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In the basidiomycete *Coprinopsis cinerea*, the stipe lifts the pileus (cap) approximately 10 cm in the final stage of fruit body development. Stipe elongation of this fungus is virtually due to elongation of stipe cells. The *elongationless1* (*eln1*) mutations affect elongation of stipe cells and veil cells. We have identified the wild-type *eln1* gene as a genomic fragment that complements the *eln1- 2* recessive mutation, and found that it encodes a yeast Cdc3-like septin. Northern analysis revealed that in the wild type the *eln1* gene is expressed only faintly in vegetative hyphae but highly expressed in elongating stipe cells. To examine subcellular localization of Eln1, we fused the enhanced green fluorescent protein (EGFP) to Eln1. EGFP signals were observed as abundant filamentous structures in cortical regions of elongating stipe cells. As stipe cells elongate, the EGFP signals are dispersed to cytoplasm and disappear. In vegetative hyphae, EGFP signals were observed only in apical cells as three types of structure: one or two dots at the hyphal tip, pellets or sticks in the cytoplasm.

317. Comparitive analysis of *fluG* function in *Aspergillus nidulans* and *Neurospora crassa*

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Asexual reproduction, conidiation, is an important phase in the life cycle of fungi. In *Aspergillus nidulans* the process initiates with the emergence of an aerial conidiophore stalk, followed by the development of an elaborate multicellular conidiophore that produces uninucleate haploid spores called conidia. Genetic regulation of conidiation is well characterized in *A. nidulans*, but comparative analysis of the function of these genes in other fungi is limited. In *A. nidulans fluG* is involved in generation of an extracellular signal required for activation of asexual development. In *Neurospora crassa*, deletion of the *fluG* ortholog (NCU04264) produced a mutant that displays no detectable phenotype. Surprisingly we found that complementation with the NCU04264 gene restored the conidiation defect of the *A. nidulans fluG* mutant. Overexpresion of NCU04264 in *A. nidulans* via the *alcA* promoter also conferred the ability to initiate conidiation in submerged culture, a phenotype similar to that exhibited by the *A. nidulans* overexpression strain in published studies. Our results demonstrate conservation of biochemical function of the *fluG* product between the two species, even though the *fluG* ortholog apparently is not required for conidiation in *N. crassa*.

318. Complementation of the *Aspergillus nidulans wetA* mutant with the apparent *Neurospora crassa* ortholog NCU01033

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The asexual life cycle of *Aspergillus nidulans* begins and ends with the production of uninucleate haploid spores called conidia. *A. nidulans* genes *brlA*, *abaA* and *wetA* are considered key regulators of conidiophore development and conidium maturation. In *A. nidulans wetA* is required for synthesis of cell wall layers that make spores impermeable. The *wetA* mutant of *A. nidulans* displays defective conidia that fail to mature and later undergo lysis. The *Neurospora crassa* deletion mutant of the *wetA* ortholog (NCU01033) does not show any defect in conidiation. This study assesses the ability of these genes to cross complement. A chimeric fusion construct with the *A. nidulans wetA* promoter and the *N. crassa* NCU01033 coding sequence restored the conidiation defect of the *A. nidulans wetA* mutant. Complementation analysis of *wetA* mutant with *N. crassa* NCU01033 expressed by its endogenous promoter is ongoing.

319. Cross-species complementation of *Aspergillus nidulans* conidiation regulators with orthologs from *Neurospora crassa*

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Conidiation is a crucial process for survival and dissemination of filamentous fungi. To study functional conservation of general regulators for conidiation, we are comparing two model systems, *Aspergillus nidulans* and *Neurospora crassa*. Three *A. nidulans* mutants (*abaA, medA*, and *stuA*) were tested by transforming with the orthologs from *N. crassa*. The *abaA* ortholog of *N. crassa* has little sequence similarity (34%), but is likely the ortholog of *A. nidulans abaA* with microsynteny. In contrast, *N. crassa acon-3* and *ama-1* have higher similarity with *A. nidulans medA* and *stuA* (51% and 60%). Expression of the *N. crassa abaA* ortholog by its endogenous promoter or via an *alcA* promoter in the *A. nidulans abaA* mutant did not complement the developmental phenotypes. Over-expressed *acon-3* and *ama-1* via *alcA* promoter partially complemented conidiophore morphology of the *A. nidulans* mutants. Expression of *acon-3* and *ama-1* by their native promoters fully complemented conidiophore morphology and conidiation of *A. nidulans* mutants. GFP-labeled AbaA, MedA and StuA localized in nuclei in *A. nidulans* live cells. These results suggest the function of conidiation regulators is both conserved (MedA and StuA) and diverged (AbaA) in two filamentous fungi.

320. Partner discrimination in yeast mating

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Saccharomyces cerevisiae cells exist in two haploid mating types **a** and **alpha**, which can fuse with each other to form diploid, **a/alpha** cells. The two mating types sense each other's presence by reciprocal sets of pheromones and pheromone receptors, with **a** cells secreting a-factor and responding to alpha- factor, and **alpha** cells secreting alpha-factor and responding to a-factor.
Beyond the receptors, the signaling pathways are identical in both mating types, but the small peptide pheromones are asymmetric, with alpha-factor-like pheromones being unmodified small peptides and a-factor-like peptides displaying the typical farnesylation CAAX box; this modification is expected to significantly increase the pheromone's hydrophobicity. This asymmetry is conserved across the Ascomycota phylum.
The pheromones allow the detection of a mating partner's presence and direction, and activate the mating response by binding to their corresponding specific receptors on the cell surface. Using a combination of computational, theoretical, and experimental techniques we are trying to understand why this asymmetry has evolved and its possible role in helping yeast cells detected gradients and discriminate between mating partners.

321. The design of genetic screens to study the molecular basis of microtubule-based cargo transport.

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In eukaryotic cells, molecular motors transport cellular material, such as organelles, chromosomes and mRNAs, by moving along the microtubule (MT) cytoskeleton. MT motors fall into two classes: dynein, which transports cargo towards the MT minus-end (from the cell periphery towards the nucleus) and kinesin, which generally moves cargo in the opposite direction. While much is known about how these motors work *in vitro*, much less is known about how these motors move cargo in cells and how these events are regulated both spatially and temporally. We are designing genetic screens in *Aspergillus nidulans* to identify factors that are required for MT-based transport. *A. nidulans* is an ideal model system for studying this problem because it has four MT-based motors that function in transport, in addition to having a sequenced genome, a high efficiency of homologous recombination, and a life-cycle that is suitable for high- throughput screening. As a basis for our screens we are identifying all organellar cargo transported by each of the four motors; we expect cargo to mislocalize in the background of motor deletions. As a starting point for identifying cargo we are tagging all of the Rab GTPases in the *A. nidulans* genome with EGFP in strains lacking each of the four motors. After identifying organelle misdistribution phenotypes we will perform genome- wide high-throughput microscopy-based screens to identify novel components required for dynein and kinesin-based transport. We expect to identify new conserved paradigms about the mechanism of cargo transport as well as some processes that will be specific to the *Aspergilli*, which could serve as therapeutic targets for fungal infections.

322. A putative eisosomal protein, SurA is involved in development of *A. nidulans*

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Eisosomes are immobile protein complexes at the plasma membrane, which mark sites of endocytosis in *Saccharomyces cerevisiae*. Sur7, Pil1 and Lsp1 are components of eisosomes. Sur7 is also a multicopy suppressor of mutations in *rvs167* that encodes an actin binding protein. Deletion of *sur7* altered sphingolipid metabolism and ascospore production in yeast. Sequence analysis showed that *A. nidulans* had one putative ortholog of Sur7 (27% identity and 47% similarity to Sur7 in *S. cerevisiae*). We generated a deletion mutant of *surA*, and this mutant displayed radial growth defect that was more severe under a restrictive temperature. In addition, the *surA* mutant showed reduced production of asexual spores, but increased sexual development including Hülle cells and cleistothecia. FM4- 64 uptake and filipin staining did not exhibit significant differences between the *surA* knockout mutant and wild type when they were grown at a permissive temperature. Characterization of the mutant at a restrictive temperature is under way. A SurA::GFP fusion protein localized at immobile cortical patches consistent with localization in yeast. Our results suggest that a putative component of endocytic eisosome is associated with development of *A. nidulans*.

323. The cell end marker protein TeaC regulates septation in *Aspergillus nidulans*

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Polarized growth in filamentous fungi depends on the correct spatial organization of the microtubule (MT) and actin cytoskeletons. In *Schizosaccharomyces pombe* it was shown that the MT cytoskeleton is required for the delivery of so called cell end marker proteins e.g. Tea1 and Tea4 to the cell ends. Here they recruit several proteins required for polarized growth, one of which is a formin which catalyzes actin cable formation. Tea4 is especially necessary for New End Take Off (NETO), which is the initiation of bipolar growth after monopolar cell growth. Latest results suggest that this machinery is conserved from fission yeast to *Aspergillus nidulans*. Here, we have characterized, TeaC,a putative homologue of Tea4. Sequence similarity between TeaC and Tea4 is only 12.5 %, but they both share a SH3 domain in the N-terminal region. Deletion of *teaC* led to an increase of the number of septa and abnormal positioning of septa. The protein localized to hyphal tips and at forming septa. TeaC interacted with the cell end marker protein TeaA (Tea1) at hyphal tips and with the formin SepA at hypal tips and at septa. Interestingly, overexpression of *teaC* repressed septation and caused the abnormal swelling of conidia. Those results suggest here the cell end marker protein TeaC regulates septation in *A.nidulans*.

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324. Assessing the roles of striatin orthologs in fungal growth, development and virulence

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Proteins of the striatin family contain a caveolin binding domain, a coiled-coil motif, and a calmodulin binding domain in the N-terminus and a WD40 repeat domain in the C- terminus. Three members of the striatin family, striatin, SG2NA and zinedin, have been identified in multicellular animals while filamentous fungi encode only one homolog. In mammals, they are mainly expressed in neuronal somatodendrites and have been characterized as cytosolic and membrane-bound proteins which play roles in signal transduction and vesicular trafficking. The striatin orthologs in filamentous fungi are associated with virulence of maize stalk rot and perithecium development in *Fusarium verticillioides* (*Fvfsr1*), and *F. graminearum* (*Fgfsr1*). In *Sordaria macrospora*, PRO11 is membrane bound and associated with sexual development. We generated *Cgfsr1* deletion mutant in *Colletotrichum graminicola*, the maize stalk rot and leaf anthracnose pathogen, to characterize its roles. The mutant showed reduced growth and reduced conidiation. The virulence of the mutant will be discussed. Additionally, the *fsrA* deletion mutant in *Aspergillus nidulans* also showed restricted colony growth with red pigment accumulation and altered sexual development. The subcellular localization of a fsrA::GFP fusion in *Aspergillus nidulans* was consistent with localization to the endoplasmic reticulum and the nuclear envelope. The detailed characters of mutants and further experimental identification of the subcellular localization will be disscussed.

325. SBF/MBF activity only partially mediates the G1/S transition in *C. albicans* and influences mating type, the mating response and opaque cell formation.

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Cells commit to mitosis and growth or follow different developmental pathways during G1 phase of the cell cycle. In *S. cerevisiae*, the CDK Cdc28p and G1 cyclin Cln3p control passage through the G1/S transition by activating the transcription factor complex SBF/MBF, which is composed of the regulatory subunit Swi6p and the DNA-binding elements Swi4p (SBF) or Mbp1p (MBF). SBF/MBF in turn activates transcription of numerous genes to allow passage through START. Regulation of the G1/S transition in *C. albicans* is poorly understood, but we previously demonstrated that a Cln3p homologue is essential for the process, and also linked to development of hyphae. In order to elucidate the regulation of the G1/S transition and determine if Cln3p activity is mediated by SBF/MBF in *C. albicans*, we investigated homologues of *SWI4, SWI6*, and *MBP1*. Deletion of *MBP1* did not influence cell morphology or growth, but deletion of *SWI4* or *SWI6* resulted in a pleiotropic phenotype, including some enlarged, oval-shaped yeast that resembled opaque cells. Surprisingly, absence of both Swi6p and Swi4p did not produce a G1 arrest, but had a synergistic effect on morphology, resulting in enlarged yeast and a higher proportion of filamentous cells. Cells lacking Swi4p and Mbp1p also did not arrest in G1 phase, but resembled *swi4Delta-* cells. Transcription profiles of the double mutants only partially overlapped with Cln3p-depleted cells. Thus, Mbp1p does not play a significant role in regulating growth, and SBF does not mediate all of Cln3p function, suggesting that the G1/S regulatory circuit in *C. albicans* involves unique features. Intriguingly, transcription profiles of *swi4Delta-, mbp1Delta-* cells revealed strong induction of mating response genes, including *MATa2, CAG1, STE18, CEK2* and *CEK1*, consistent with the opaque appearance of some cells. The *swi4Delta-, mbp1Delta-* cells were also found to be homozygous at the mating type locus (MTL), which is in contrast to the heterozygous state in white cells, and normally requires chromosomal rearrangements to be achieved. Thus, SBF/MBF activity may also contribute to the regulation of mating type, the mating response, and possibly differentiation of the opaque state.

326. Cell surface organization in the wheat pathogen *Fusarium graminearum*.

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The ascomycete fungus *Fusarium graminearum* causes fusarium head blight of wheat and barley. We are currently utilizing several approaches to assess the importance of the cell surface in growth and virulence of *F. graminearum*. First, we inventoried all predicted glycosylphosphatidylinositol (GPI)-anchored proteins using the publicly available big-Pi fungal predictor. Our search yielded 205 predicted proteins, 147 of which also had a predicted signal peptide, further supporting their localization to the cell surface. Most (90) did not have a predicted function when primary sequence was compared against the major databases. Of the proteins that did have a predicted function, most were carbohydrate-modifying enzymes likely involved in cell wall modeling during fungal growth. We generated knockout mutants of a supoxide dismutase-like protein, and two proteins of unknown function containing internal genetic repeats. None of the mutants generated deviated from wildtype in growth or virulence on wheat heads. Our second approach was to target a protein encoding a ceramide synthase (Bar1=FGSG_09423.3). By doing so, we aimed to reduce sphingolipid production and hence disrupt sphingolipid-rich membrane rafts. The *bar1* deletion mutant displayed a severe growth phenotype on all media tested, and failed to form macroconidia unless supplemented with 4% NaCl. The macroconidia displayed an aberrant shape, reduction in size, and fewer cells per conidium. The germlings of *bar1* exhibited wide, misshapen germ tubes compared to long, slender germ tubes of wildtype. Also, when sterol-rich region were examined using filipin, *bar1* germ tubes failed to stain brightly at the tip as is seen in wildtype hyphae. *bar1* mutants also were also more sensitive to cell wall disturbing agents calcafluor white and congo red, suggesting an altered cell wall. Although these severe phenotypes were observed, *bar1* mutants were still able to differentiate infection hyphae when germinated on wheat glumes.

327. Role of the small G-protein Ras on sexual development in the homobasidiomycete *Schizophyllum commune*

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The involvement of the small monomeric G-protein Ras1 and the GTPase-activating protein for RasGap1 in sexual development and cell morphogenesis were investigated in the white rot fungus *S. commune*. To analyse the function of Ras1, amino acid mutations in the GTP-binding region were inserted *in vitro*. The phenotypes of the transformants carrying constitutive active alleles Ras^{G12V} and Ras^{Q61L} were compared with *gap1* deletion strains. Disruption of *gap1* leads to accumulation of Ras in its activated, GTP-bound state and to constitutive Ras signaling. Microscopic examinations showed that constitutive active Ras1 and *Dgap1* strains exhibited a disorientated growth pattern on solid media. The hyphae of the Ras1 mutants show a hyperbranching effect. Constitutive Ras1 mutant strains are unable to form dikaryons in compatible mating interactions but the mating of Ras1 mutant strains with compatible wildtype strains generates normal fruiting bodies. The germination rate of the spores is reduced in comparison to wildtype spores. Homozygote *Dgap1/Dgap1* dikaryons produce abnormal fruit bodies with abnormal gills and without spore production. Investigation of the cAMP-dependent protein kinase A showed that constitutive active Ras1 and *Dgap1* mutant strains exhibit a strong increase of the activity of the PKA. This points at a Ras1/cAMP mediated signal transduction pathway in *S. commune*.

328. Analysis of SONB, a nuclear pore complex protein involved in the DNA damage response.

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Nuclear pore complexes (NPCs) are composed of a set of proteins called nucleoporins (Nups) and allow the transport of proteins and nucleic acids across the nuclear envelope. Some nucleoporins appear to have additional functions aside from their role in transport. For example, components of the NUP84 subcomplex have previously been implicated in the response to DNA damage. NPCs have been associated with telomere anchoring/silencing, and telomere anchoring to the nuclear periphery is essential for efficient repair of DNA damage in subtelomeric regions. SONB^{Nup98} is a conserved component of nuclear pore complexes and is essential in *Aspergillus nidulans*. The *sonB1* allele was identified in a screen for suppressors of the temperature sensitive *nimA1* allele of the mitotic NIMA kinase. Importantly, subsequent genetic analyses indicated SONB has a role in a novel response to DNA damage. *sonB1* mutant cells are hypersensitive to DNA damaging agents and show synthetic genetic interactions with *scaA*^{Nbs1}, a breast cancer susceptibility gene. Mutations in NBS1 cause an increased sensitivity to DNA damage and shortened telomeres. We have extended these studies and found that *sonB1* shows synthetic genetic interactions with the same components of the NUP84 subcomplex previously implicated in the DNA damage response in yeast. We will examine the role of SONB in telomere anchoring and activation of a DNA damage checkpoint, and partially map out this novel DNA damage response pathway that involves components of the nuclear pore complex.

329. A newly discovered role for cell-cycle regulator Cdc14 in basal body function suggested by studies in *Phytophthora infestans*.

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Functions of Cdc14 orthologues reported to date have involved regulating various aspects of mitosis such as mitotic exit and cytokinesis. The *Phytophthora infestans* ortholog (PiCdc14) complements a yeast Cdc14 mutation, suggesting a role in mitosis. However, PiCdc14 is normally expressed only in spores. Moreover, P. infestans transformants overexpressing PiCdc14 exhibit normal nuclear behaviour, growth, sporulation, and germination, which suggests that it might not act in mitosis. To further explore its function, transformants expressing PiCdc14 tagged N-and C-terminally with GFP were analysed. Despite some heterogeneity amongst spores, a trend in the subcellular localization of PiCdc14 during development was discerned. PiCdc14 is distributed throughout the cytoplasm in undifferentiated sporangia. In chilled sporangia and zoospores, PiCdc14 tends to accumulate next to nuclei in an organelle which appears to be the basal body (the microtubule nucleation site from which flagella develop). This suggests a model whereby the ancestral role of Cdc14 involved the function of the centrosome, which in many species regulates aspects of mitosis. In some eukaryotes this role diversified to regulate basal bodies, which are related to centrosomes.

330. SH3-harboring proteins contribute to fast polar growth of the filamentous fungus *Ashbya gossypii*

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Endocytosis is a dynamic process involving the ordered recruitment of proteins: components of eisosomes, clathrin, endocytic adaptors, actin nucleation promoting factors, and amphiphysins. Endocytic proteins possessing SH3 (src homology 3) domains are believed to be responsible for the assembly of the endocytic vesicles at the plasma membrane by interacting with other proteins of the endocytic complex. We tagged the endocytic myosin protein *Ag*Myo3/5 with EGFP to visualize of the endocytic patches *in vivo* and measured the lifetime of *Ag*Myo3/5-EGFP fluorescence in seven deletion backgrounds of SH3-domain genes. The deletion of *AgABP1* and *AgSLA1* showed an aberrant endocytic localization pattern and *Ag*Myo3/5- EGFP lifetime. In *AgSLA1deltaAg*Myo3/5-EGFP strain, growth of the fungus decreased by 50%. Endocytic patches in the reference strain were most concentrated within 10 micrometers from the hyphal tips, but in the *Agabp1deltaAg*Myo3/5-EGFP strain, *Ag*Myo3/5-EGFP patches were located further subapically. We further deleted the SH3-domain in *AgABP1* and found that the *Ag*Myo3/5-EGFP fluorescence dynamics recovered, but the fluorescence remained concentrated subapically. The results show that the SH3 domains of *Ag*Abp1 and *Ag*Sla1 play a role in the spatial arrangement of proteins involved in endocytosis in *A. gossypii*.

331. Characterization of Protein *O*-mannosyltransferases and their client proteins in *Aspergillus nidulans*

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Protein *O*-mannosylation is important for normal structure, function, half-life and immunogenicity of proteins. In eukaryotes, protein *O*-mannosylation initiates at the endoplasmic reticulum where the first mannose residue is transferred to the hydroxyl group of serine or threonine residues of target proteins co- and post-translationally by the activity of protein *O*-mannosyltransferases (PMTs). PMTs are found in bacteria, fungi, and animals but are not present in plants. In fungi, PMTs are divided into three subfamilies, PMT1, PMT2 and PMT4 and each species has 3-7 PMTs. *Aspergillus nidulans* possesses three PMTs, Pmt1, Pmt2 and Pmt4. In the current work we show that single *pmt* deletion mutants are viable and temperature sensitive. The *delta pmt1 delta pmt2* double mutant is the only viable double mutant and has additive phenotypes of *delta pmt1* mutant and *delta pmt2* mutant. The triple mutant is lethal. Lower temperatures and osmoticum partially restore wildtype hyphal growth and conidiation of these *delta pmt* mutants. Chemicals disturbing cell wall synthesis alter growth of mutants suggesting that protein *O*-mannosylation is important for cell wall integrity of *A. nidulans*. AN5660 is an ortholog of Wsc1p, an *S. cerevisiae* stress receptor important in the cell wall integrity pathway. We found that AN5660 is mannosylated by Pmt2.

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332. Biological functions of the motor protein dynein in the basidiomycete *Schizophyllum commune*

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The filamentous basidiomycete *Schizophyllum commune* is a model organism for investigating sexual development: this fungus is able to complete its whole life cycle on minimal medium and is characterized by a tetrapolar mating-system with multi-allelic incompatibility factors allowing the formation of thousands of mating types. This work is focusing on the cytoskeleton of *S. commune* and on dynein, the motor protein- complex for transport along microtubules. Dynein consists of two heavy chains, several intermediate chains, four light intermediate chains and several light chains. The genes *dhc1* and *dhc2* encode together the dynein heavy chain. *Dhc2* knockout mutants differ from the wildtypestrains in a reduced growthrate as well as changes in nuclear positioning. The aim of this work is to breed strains of *S. commune* which have a deletion in the gene *dhc1* to test whether a specific function can be assigned to each Dhc protein. A feasible function could be that Dhc1 is responsible for spindle assembly and spindle orientation during cell division, or for chromosomal movement during mitosis. In fungi it is also required for nuclear migration. Using immunfluorescence staining for Dhc1 and Dhc2, the localization of both proteins was examined. This project is supported by the Deutsche Forschungsgesellschaft (DFG).

333. Imaging living hyphae of *Sordaria macrospora*

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The filamentous ascomycete *Sordaria macrospora* is a model system for eukaryotic cell differentiation, because it forms complex fruiting bodies consisting of a number of different cell types. To gain insight into the mechanisms of fruiting body formation, we have established different microscopic techniques for *S. macrospora*. First, we have tested standard fluorescent proteins like EGFP, EYFP and DsRed, and novel fluorescent proteins like mKalama1, EBFP2, tdTomato and mCherry. We have further employed a codon-adapted mRFP1 protein. Bimolecular fluorescence complementation has recently been developed for filamentous fungi, and first results using this method in *S. macrospora* will be presented. Second, we have targeted fluorescent proteins to different organelles by fusing them to organellar target sequences or organelle-localized proteins. Examples are ER-targeted DsRed or EGFP and histone fusion constructs with EYFP and ECFP. These enabled us to dissect organelle morphology in different developmental mutants of *S. macrospora*, and to analyze hyphal fusion in different strains. Furthermore, we performed co-localization studies with labeled developmental proteins and organellar markers. Third, we have established a live cell imaging system for *S. macrospora*. The system is based on an upright microscope and utilizes a heating plate with small Petri dishes. So far, we have been able to follow ascospore germination and ascogon formation in *S. macrospora*.

334. Disassembly of septin filaments during transition from collar to ring-like structures

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Septins are conserved GTP-binding proteins essential for cytokinesis in animal and fungal cells. The filament-forming septins were discovered in the yeast *Saccharomyces cerevisiae* where they undergo a complex structural reorganization during budding growth. Septin filaments align in longitudinal orientation along the mother-bud axis and form an hourglass- or collar-like structure. During cytokinesis the septin collar is converted into two rings with circumferential orientation of filaments. The molecular mechanism of this dynamic structural transition is yet unknown. We could show that septin collar-to-ring transition in budding cells of the dimorphic fungus *Ustilago maydis* involves disassembly and reassembly of septin filaments. We used a chemical genetic approach to arrest dividing cells with a stable septin collar. We observed that at this stage the essential myosin light chain Cdc4 but not the FCH domain protein Cdc15 is associated with the septin collar. Inhibitor release results in instantaneous disassembly of the septin collar, while Cdc4 recruits Cdc15 from the cytoplasm to form the contractile actomyosin ring. Reassembly of septin filaments into a ring-like structure occurred upon constriction of the actomyosin ring. We propose that septin filaments exert a dual function in budding cells. Septin filaments in longitudinal orientation confer mechanical stability of the mother-bud neck while circumferential orientation of septin filaments is required for their function during cytokinesis.

335. The *Aspergillus nidulans* orthologue of protein kinase C (PkcA) localizes to forming septa in a formin- and actin-dependent manner.

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The *calC*2 mutation in the *Aspergillus nidulans* orthologue of protein kinase C (PkcA) causes hypersensitivity to Calcofluor White along with other drug sensitivities which indicate a defect in cell wall integrity. Placing the PkcA gene under the control of the regulatable AlcA(p) promoter severely compromises hyphal morphogenesis and resistance to wall damaging drugs under repressed conditions. A PkcA::GFP chimera localizes to hyphal apices and growing septa, as well as to the conidiogenous apices of phialides. These observations indicate that PkcA plays roles in polarized wall growth, in cytokinesis, and in the cell wall integrity pathway. In order to investigate the mechanisms of PkcA targeting to hyphal tips and points of septation, we exposed a strain expressing PkcA::GFP under its native promoter to the F-actin disrupting agent cytochalasin A. After 15 minutes exposure, PkcA failed to localize to cortical rings (marking sites of incipient septation) or to hyphal apices. Instead, PkcA localized only to scattered cytoplasmic patches, possibly representing detachment of already formed contractile rings. In addition, we have generated a strain expressing Pkc::GFP in a temperature-sensitive *sepA*1 background (*A. nidulans* formin orthologue). When grown for several hours under restrictive conditions, PkcA still localized in the normal manner to hyphal apices, but no cortical rings were formed. This indicates that, even though PkcA and SepA occur together at both hyphal apices and septation sites, the relationship of PkcA to SepA is different at the two locations.

336. The *Aspergillus nidulans* kinesin-3 UncA motor moves vesicles along a subpopulation of microtubules

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The extremely polarized growth form of filamentous fungi imposes a huge challenge on the cellular transport machinery, as proteins and lipids required for hyphal extension need to be continuously transported to the growing tip. Recently, it was shown that endocytosis is also important for hyphal growth. Here we found that the *Aspergillus nidulans* kinesin-3 motor protein UncA transports vesicles along microtubules (MTs) and is required for fast hyphal extension. Most surprisingly, UncA-dependent vesicle movement occurred along a subpopulation of MTs. The MT cytoskeleton is characterized by its dynamic instability. In addition, MTs can be made up of different tubulin isoforms and of different post-translationally modified tubulins, such as acetylated or detyrosinated tubulins. GFP labelled UncA^{rigor} decorated a single MT, which remained intact during mitosis, while other cytoplasmic MTs were depolymerised. Mitotic spindles were not labelled with GFP-UncA^{rigor} but reacted with a specific antibody against tyrosinated alpha-tubulin. Hence UncA binds preferentially to detyrosinated MTs. In contrast, kinesin-1 (conventional kinesin, kinA) and kinesin-7 (KipA) did not show a preference for certain MTs. This is the first example for different MT subpopulations in filamentous fungi and the first example for the preference of a kinesin-3 motor for detyrosinated MTs.

337. A Couple of Clocks in *Neurospora crassa*: Strength in numbers?

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According to recent insights, the cellular circadian clock in all organisms consists of a network of coupled molecular oscillators. In *Neurospora crassa*, in the absence of the FRQ-based negative transcription-translation feedback loop, strains still systematically entrain to temperature. Also, they retain circadian oscillations in the expression of nitrogen assimilation pathways, e.g. nitrate reductase activity and amino acid biosynthesis. The FRQ-based feedback loop therefore acts as a non-essential sub- system, perhaps a pacemaker oscillator, of the circadian clock of *N. crassa*. We propose to use *N. crassa* as a model system to explore how a multi-oscillator network structure, as determined by coupling or connectivity, impacts the system as a whole. We ask how circadian properties such as entrainability and robustness emerge from the network construction. We use novel gene reporter tools to track components of the different molecular oscillators in *N. crassa*, as a system for investigating the effect of coupling on these properties.

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Biochemistry and Metabolism

338. Characterization of the histone deacetylase *MoHDA1* in *Magnaporthe oryzae*

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Epigenetic regulation of gene-expression is thought to play important roles in the cellular processes such as development and metabolism. The relationships between epigenetic regulation and fungal development are poorly understood. Recently, it was reported that knockout of a class II histone deacetylase (HDAC) gene, *hdaA*, in *Aspergillus nidulans* caused a transcriptional activation of subtelomeric secondary metabolic gene clusters and that the mutants showed a remarkable reduction of growth under conditions of oxidative stress. The rice blast fungus, *Magnaporthe oryzae*, is known to require alterations of global gene-expressions at the pre-penetration stage. Fungal secondary metabolites, such as melanin and *ACE1* cluster product, are thought to play important role for virulence. We characterized the roles of a class II HDAC, *MoHDA1*, in morphogeneses and metabolisms of *M. oryzae*. In contrast to the unaltered morphology of *A. nidulans* by deletion of this gene, the *Mohda1* deletion mutant showed reduced conidiation and vegetative growth and induced melanin biosynthesis. An HDAC inhibitor, trichostatin A, also induced melanin biosynthesis. Our results suggested that MoHda1 may function in regulation of global cellular process such as conidiation and metabolism, and that HDAC activity was important for melanin biosynthesis in *M. oryzae*.

339. Fenhexamid resistance in the phytopathogenic fungus, *Botrytis cinerea*

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Fenhexamid is one of the most active fungicide used against *Botrytis cinerea* on vineyards. Sensitivity monitoring showed occurrence of three phenotypes of resistant strains to fenhexamid when tested in laboratory. These three phenotypes, called HydR1, 2 and 3, are highly resistant at mycelium growth stage. Molecular characterisation of these strains will allow a better understanding of *B cinerea* resistance mechanisms towards the fungicide. Biochemical experimentations (Debieu et al., 2001), suggest the 3 keto reductase (erg27) from the C4 demethylation process of ergosterol biosynthesis as a target for this compound. Sequence analysis, enzyme inhibition measurements and reverse genetics (Fillinger et al., 2008) showed mutations in the *erg27* gene in the HydR3 strains. An inducible RNAi approach was initiated for the functional analysis of the *erg27* gene in B.cinerea and to validate this enzyme as target of fenhexamid. In addition, a site- directed mutagenesis approach of the *erg27* gene was carried out in the fenhexamid sensitive *B05.10 ?ku70* strain in order to evaluate the effect of each mutation on fenhexamid susceptibility and on the enzyme affinity for the compound. Methodology of the gene replacement and first results will be presented.

340. Cell wall biosynthesis in Oomycetes.

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Oomycetes have long been considered as a separate class within the kingdom Fungi. However, sequence and phenotypic analyses indicate that they are closer to brown algae and taxonomically unrelated to true fungi. They are currently classified in the Stramenopile kingdom together with heterokont algae and water molds. The most important features that distinguish Oomycetes from true fungi is their specific cell wall composition. The latter consists essentially of (1→3)-beta-glucans, (1→6)- beta-glucans and cellulose whereas chitin, a major cell wall component of fungi, occurs in minute amounts in the walls of some Oomycetes. In addition to saprophytes that are beneficial to natural ecosystems by contributing to the recycling of nutrients from organic decayed matter, the Oomycete phylum comprises plant or animal pathogens responsible for severe environmental damage and economic loss. Cell wall carbohydrate synthases are vital for Oomycetes and represent potential targets for specific inhibitors. However, they have not been characterized and their molecular mechanisms are poorly understood. This presentation will summarize our latest results on the biosynthesis of cellulose, (1→3)-beta-D-glucan and chitin in the Oomycete *Saprolegnia monoica*. In particular, the isolation and characterization of new families of genes encoding cell wall carbohydrate synthases and the significance of our recent discovery that (1→3)-beta-D-glucan and chitin synthases of Oomycetes are located in lipid rafts will be discussed.

341. ICARUS a protein involved in the dormancy of ultra stress-resistant ascospores.

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Ascospores of *Talaromyces macrosporus* are the most resilient eukaryotic structures described to date and survive ultrahigh pressure (6000 Bar), temperature (85 °C) and drought. They exhibit constitutive dormancy and are, for example, activated to germinate after a heat treatment at 85 ºC. Upon activation, changes in the permeability and structure of the thick ornamented cell wall were observed with different techniques. Activation by high temperature was associated with a release of large amounts of a small (7 kD) dominant protein (≈ 5% of the total cell protein) from the cell wall. Deletion mutants show a clear decrease of the dormancy of the spores which is correlated with permeability changes of the cell wall. The unusual aggregation properties of the protein suggest that it acts as a seal that isolates the cell of its environment. Removal of the seal might break the dormancy of the ascospores.

342. Regulation of extracellular pH by *Candida albicans*

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pH homeostasis is critical for all organisms; pathogens, in particular, are confronted with a wide range of pH conditions at different body sites. In *Candida albicans*, the most important fungal pathogen, adaptation to acidic versus neutral pH has been shown to be critical for virulence in distinct sites (mucosal versus systemic, for instance). Beyond this, however, some fungi have been shown to actively change the pH of their environment. We have shown that *C. albicans* grown in specific conditions, namely starved for glucose but with ample amino acids, can make dramatic changes to extracellular pH – neutralizing a culture from pH 4 to ~7 during overnight growth. This phenomenon is completely repressed by glucose. Genetic analysis has identified components of intermediate carbon metabolism that are required for neutralization. These include the acetyl-CoA hydrolase (ACH1) and a family of putative acetate transporters (ATO), each of which abrogate pH changes when mutated. Microarray analysis of cells undergoing alkalinization reinforces the idea that this is a byproduct of nutrient starvation, a condition that has been linked with virulence, and we have validated a wide variety of candidates identified by array. We propose a model in which *C. albicans* co-opts alternative carbon metabolism to excrete basic compounds, including ammonia, in order to raise environmental pH, stimulate morphogenesis, and inhibit host acid hydrolases, thus directly contributing to pathogenesis.

343. A systems biology approach towards improvement of itaconic acid production in Aspergillus sp.

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Filamentous fungi are widely used for enzyme and metabolite production for a wide variety of uses – for example food, feed, textile, paper and pulp, fuels and chemicals, detergents - due to the development of extremely productive strains and production processes. With the advent of molecular genetic tools, in the last two decades very successful strain improvement programs for protein and secondary metabolites have been developed. However, for primary metabolites, such as organic acids, which are still by volume the largest fungal bioproducts, real breakthroughs have not been made in recent years. In our research we have addressed the production of one of the commercially interesting building-block organic acids, itaconic acid. To unambiguously identify the itaconic acid biosynthetic pathway several parallel approaches were taken using *Aspergillus terreus* as parental host strain. Using a combination of controlled fermentation design, reversed genetics and transcriptomics approaches the pathway specific cis-aconitate decarboxylase (CAD) encoding gene was identified. This gene encodes the enzyme required to make itaconic acid from general TCA cycle intermediates. In addition to this gene several other target genes for improvement of itaconic acid production were identified. A highly relevant issue for the bioproduction of bulk-products like organic acids is also the cost of production. As production costs rely for 30-60% on the cost of feedstock, in frame with our itaconic acid project we also evaluated performance and feedstock versatility of several potential microbial production hosts on lignocellulosic substrates. From this analysis *Aspergillus niger* was selected as preferred host.

344. Processing of amatoxin and phallotoxin proproteins by prolyl oligopeptidase from *Conocybe albipes*

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The cyclic peptide amatoxins (eight amino acids) and phallotoxins (seven amino acids) of poisonous *Amanita* mushrooms are encoded by small genes encoding predicted proproteins of 35 and 34 amino acids, respectively. The toxin regions of the proproteins are flanked by highly conserved proline residues. Prolyl oligopeptidases (POPs), enzymes that specifically cleave small peptides after proline residues, are thus candidates to be involved in the initial proteolytic processing of the toxin peptides. *Conocybe albipes* is a mushroom, commonly found in lawns, that produces the phallotoxin called phallacidin, but not amatoxins. We have purified the POP protein from *C. albipes* and determined its peptide cleavage activity by HPLC, MS and MS/MS. The results indicate that *C. albipes* POP can process a synthetic 15-mer phallacidin precursor by cleaving immediately following the two proline residues flanking the phallacidin peptide; one proline is thereby removed and one proline remains in the mature 7-mer. These results are consistent with POP catalyzing the first processing step in phallotoxin biosynthesis in vivo. *Conocybe* POP also shows substrate specificity based on the fact that it processes a phallacidin precursor more efficiently than an amanitin precursor.

345. Import of the antifungal fluconazole by facilitated diffusion in *Candida albicans* and other pathogenic fungi

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Very little is known concerning the import of azole antifungal drugs into pathogenic fungi. In vitro import and accumulation of radiolabeled fluconazole was examined in the pathogenic yeast, *Candida albicans* . In the absence of glucose, all strains accumulate fluconazole and display saturation kinetics, implying that import occurs via facilitated diffusion involving an ATP-independent transporter. Other azoles compete with fluconazole for import, suggesting that both triazoles and imidazoles utilize the same transporter. Fluconazole import is observed in other pathogenic fungi, including *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, and *Candida krusei*, indicating that the transporter is conserved among fungal species. Finally, import levels vary among resistant clinical isolates, suggesting that import is a previously uncharacterized mechanism of resistance to azole drugs in *Candida albicans*.

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346. Serine-type carboxypeptidases in *Aspergillus oryzae*.

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Serine-type carboxypeptidases(CPase) are able to release most amino-acid residues from the C-terminus of peptides and proteins at acidic pH. CPase genes in the genome of *A. oryzae* inferred with the active site motives. There are 11 putative genes encoding CPase. We constructed recombinant *A. nidulans* to express some of the enzymes to clarify the enzymatic properties. Several recombinant enzymes were purified and the gene products, CPases, were confirmed by peptide mass finger printing method. The purified enzymes released the C-terminal amino acid from angiotensin sequentially. The optimum pH of the enzymes for Z-Glu-Tyr around pH 4 and the enzymes were stable at pH 3 to 7. The enzymes were classified as serine-type carboxypeptidase. The specificities of individual enzymes were differed for small synthetic peptides.

This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

347. Clustered genes common to *Aspergillus fumigatus* and ergot fungi control early steps in ergot alkaloid biosynthesis.

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Ergot alkaloids, indole-derived mycotoxins, interact with multiple monoamine neurotransmitter receptors and cause disease in exposed individuals. They have been well studied in the ergot fungus, *Claviceps purpurea*, and have been reported in some closely related grass endophytes, as well as the distantly related opportunistic human pathogen *Aspergillus fumigatus*. Alkaloids produced by *A. fumigatus* differ from those of clavicipitaceous fungi. Ergot alkaloid pathways of *A. fumigatus* and ergot fungi are hypothesized to share early biosynthetic steps, diverging after the formation of the intermediate, chanoclavine. A homologue of *dmaW*, a gene encoding dimethylallyltryptophan synthase in *Neotyphodium* endophytes, was found in the *A. fumigatus* genome, where it also controls the determinate step in alkaloid production. Comparison of genes clustered around *A. fumigatus dmaW* to those clustered with *dmaW* in the ergot fungi revealed potential homologues that could encode proteins controlling early, shared steps in the pathway. Functional analyses via gene knock out of four *A. fumigatus* genes rendered mutants with altered alkaloid profiles. All of the mutants lacked alkaloids from the latter part of the pathway; one mutant accumulated chanoclavine. Mutants with altered alkaloid profiles are valuable for testing the role of ergot alkaloids in animal pathogenesis and toxicoses. Elucidation of ergot alkaloid biosynthesis, along with the capacity to control the spectrum of alkaloids produced, may be beneficial to agriculture and medicine.

348. Functional analysis of indole diterpene biosynthesis in the grass endosymbiont *Epichloë festucae*.

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Epichloë festucae in association with *Lolium perenne* synthesizes a range of secondary metabolites that confer bioprotective benefits to the grass host. One important class of metabolites found in this symbiotum is the indole diterpenes (IDs), of which lolitrem B is the most abundant. We recently identified a complex LTM (lolitrem) locus comprised of three clusters of genes for the biosynthesis of this metabolite. The clusters comprise three (ltmG, M and K), five (ltmP, Q, F, C and B) and two (ltmE and J) genes respectively. Ltm G, C, M and B are proposed to be required for the synthesis of paspaline. The objectives of this study were to determine the function of the other ltm gene products by analyzing products that accumulate in symbiota containing deletion mutants of *E. festucae*, and by feeding putative intermediates to *Penicillium paxilli* containing copies of these genes under the control of a native ID biosynthetic gene promoter. Deletion analysis suggests that Ltm E and J catalyse prenylation and oxidation steps for the formation of the A- and B-rings of lolitrem B; LtmF and K catalyse prenylation and oxidation steps for formation of ring-I; LtmP catalyses demethylation of C- 12 and hydroxylation of C-10; and LtmQ catalyses hydroxylation of C-13. Feeding studies demonstrate that LtmP can convert paspaline to PC-M6 and 13-desoxypaxilline, as previously shown for PaxP. However, LtmQ, unlike PaxQ, appears unable to utilize 13- desoxypaxilline as a substrate. On the basis of these results we will propose a scheme for ID biosynthesis in *E. festucae*.

349. Comparison of low molecular mass metallo-endopeptidases in *Aspergillus oryzae*.

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Deuterolysin (DeuA) is a low molecular mass (ca. 20kDa) metallo-endopeptidase from *Aspergilli*. Two deuterolysin homologue genes (designated as *deuB* and *deuC*) were found in *A. oryzae* genome database. The amino acid sequence identities of DeuB and DeuC against deuterolysin were shown as 67% and 31%, respectively. We constructed recombinant *A. nidulans* to express three enzymes to clarify the division of roles by the enzymes. Each ORF (*deuA, deuB, deuC*) was amplified with PCR according to the genome database of *A. oryzae*, and inserted at the downstream of amylase promoter in the expression plasmid containing *aurA* gene and *AMA1* sequence. *A. nidulans* was transformed by the constructed plasmid and each transformant was cultured with YPM medium and the expression of DeuA and DeuB was confirmed. Both enzymes showed similar properties such as substrate specificity but thermostability. The expression of DeuC was not observed under various culture conditions. It was conceivable that the incorrect splicing of the intron or non-splicing should be occurred in the expression of DeuC by RT-PCR. This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

350. Enzymatic analysis of a novel alanyl dipeptidyl peptidase (DPP V) from the koji mold *Aspergillus oryzae*.

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In *Aspergillus fumigatus*, two dipeptidyl peptidases (DPP) were isolated from and characterized. One is prolyl dipeptidyl peptidase (AfDPP IV) that released the X-Pro dipeptide, and the other is alanyl dipeptidyl peptidase (AfDPP V) that released the X-Ala dipeptides from N-terminal of peptides. One sequence of *dpp IV* and two *dpp V* were found in the *A. oryzae* genome DNA base. These genes were overexpressed in *A. nidulans* and the *A. oryzae* DPPs (AoDPP IV, AoDPP Va and AoDPP Vb) were purified from culture broth. AoDPP IV showed higher substrate specificity than that of AfDPP IV. AoDPP IV could release only X-Pro dipeptide, but AfDPP IV is reported to be able to release not only X-Pro but also Gly-Phe and lesser extent of X-Ala. On the other hand AoDPP Va and Vb could digest X-Ala-*p*NA and Gly-Phe-*p*NA similar to AfDPP V. However, the most suitable substrate of AoDPP Va was Gly-Phe-*p*NA different from AoDPP Vb and AfDPP V. Furthermore, AoDPP Vb showed prolyl dipeptidyl peptidase (DPP IV) like activities, releasing X-Pro dipeptide. As the result of those, AoDPP Vb should be classified a novel alanyl dipeptidyl peptidase familly different from DPP V. This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

351. Melanin biosynthesis genes are developmentally regulated in the homothallic ascomycete *Sordaria macrospora*

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The filamentous ascomycete *Sordaria macrospora* develops perithecia during its sexual lifecycle which is accompanied by melanin accumulation. To analyze the connection between sexual development and melanin biosynthesis, the four melanin biosynthesis genes *pks*, *teh*, *sdh* and *tih* were isolated and their homology to genes involved in 1,8 dihydroxynaphthalene (DHN) melanin biosynthesis was shown. The presence of DHN melanin in *S. macrospora* was further confirmed by disrupting the *pks* gene encoding a putative polyketide synthase and by RNA interference-mediated silencing of the *sdh* gene encoding a putative scytalone dehydratase. Because melanin occurs in fruiting bodies that develop through several intermediate stages within 7 days of growth, a Northern analysis of a developmental time-course was conducted. These data revealed a time-dependent regulation of *teh* and *sdh* transcript levels. Quantitative real-time PCR was used to compare transcript levels during vegetative and sexual development, and all four melanin biosynthesis genes are significantly down-regulated during vegetative growth. Further quantitative real-time PCR and Northern blot analysis using different developmental mutants confirmed that melanin biosynthesis is linked to fruiting body development and is under the control of specific regulatory genes that participate in sexual differentiation.

352. Withdrawn

353. Analysis of secondary metabolite gene clusters in the genome of *Epichloë festucae*

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Epichloë festucae is a model endophytic fungus for which the genome has recently been sequenced. Analyses based on protein domain structures revealed 29 clusters of biosynthetic gene models coding for known or predicted secondary metabolites in the fungal genomic sequence data. Most of the clusters contained a polyketide synthase gene, nonribosomal peptide synthetase gene, or both, although these were not featured in the loline alkaloid cluster (*LOL*). We investigated linkages between the secondary metabolite biosynthetic genes and clusters, their distribution throughout the fungal genome, and their possible telomeric associations. We conducted long-range mapping of putative secondary metabolite gene clusters and telomeres on *Not*I restriction fragments from fungal genomic DNA. Preliminary results suggested that several of the clusters are linked to form clusters of clusters. Even more intriguing, a third of the identified clusters appear to be located in subtelomeric regions. Both the ergot and loline alkaloid biosynthesis gene clusters appear to be subtelomeric. Unique for fungal genomes, we found that more than 50% of the telomeres are associated with secondary metabolite gene clusters, which may be important in regulation of pathway gene expression, or to maintain their polymorphism in populations.

354. Discovery of the emericellamide gene cluster by genomic mining in *Aspergillus nidulans*

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The recently sequenced genomes of several *Aspergillus* species, including *A. nidulans*, have revealed that these organisms have the potential to produce a surprising large range of natural products. Exploiting this discovery will, in turn, depend on advancements in tools for manipulating *Aspergillus* genomes and on understanding *Aspergillus* secondary metabolite regulation. We have employed recently developed gene targeting procedures, in combination with natural products chemistry, to discover novel nonribosomal peptide synthetase (NRPS)/polyketide synthase (PKS) gene cluster that synthesizes emericellamides. We further establish the boundaries of the eas (emericellamide synthesis) gene cluster and propose a biosynthetic pathway through a series of targeted deletions. The identification of this cluster opens the door to engineering novel analogs of these complex metabolites.

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355. Characterization and functional analysis of *suo5*, a suppressor of the *pex2* peroxisomal mutant of *Podospora anserina*.
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P. anserina pex2 mutants show both metabolic (*ie* growth defects on oleic acid) and developmental defaults (*ie* defect in karyogamy and meiosis engagement). A screening for suppressors of *pex2* mutations allowed the identification of several loci which mutations partially restore one or several of the *pex2* mutant defects [1]. Interestingly, one of these suppressors, *suo5* although harboring a growth defect on oleic acid, partially restores the *pex2* growth defaults on oleic acid, together with peroxisome biogenesis and developmental defects. By genetic mapping, *suo5* was located on chromosome 6 and linked to the *mus51* gene [2]. A new microsatellite approach [3] allowed to refine *suo5* localization on a 700kb region. After a complementation cloning approach based on the restoration of a wild-type growth on oleic acid, the *suo5* gene was characterized. Sequencing showed that the *suo5* mutation leads to a truncated protein. The deleted *suo5* strain will be constructed and functional analysis in both wild-type and *pex2* mutant backgrounds will be performed. We acknowledge the ANR (ANR- 05-BLAN-0385-01) for funding. 1. Ruprich-Robert, G, *et al.* , Genetics, 2002. 161: 1089 2. El-Khoury, R, *et al.* , Curr Genet, 2008. 53: 249 3. Espagne, E, *et al.* , Genome Biol, 2008. 9: R77

356. Catalases during the life cycle of the filamentous fungus *Podospora anserina*.
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In *P. anserina*, mutations in some peroxin genes such as *pex2* trigger sexual defects (ie the dikaryotic cells maintain a proliferative state instead of engaging into karyogamy and meiosis) [1, 2]. Previous analysis of a suppressor of the *pex2* mutations, which encodes the mitochondrial citrate synthase, has suggested that high hydrogen peroxide concentration could be responsible for the pex2 phenotypes [3]. To study the role of hydrogen peroxide during the life cycle, we cloned and invalidated the five catalase genes involved in the degradation of hydrogen peroxide was initiated. We have identified 4 monofunctional catalases and one bifunctional catalase-peroxidase in the *P. anserina* genome [4]. The expression of the catalases has been followed during the sexual development. The five genes have been deleted and we constructed various mutant strains ranging from double mutants to a mutant strain completely devoid of catalase. Characterization of these strains is under investigation. The results as a whole will be discussed in the regard to the role of hydrogen peroxide throughout the fungus life cycle. We acknowledge the ANR (ANR-05-BLAN-0385-01) for funding.
1. Berteaux-Lecellier, V, *et al.*, Cell, 1995. 81: 1043 2. Peraza-Reyes, L, *et al.*, Traffic, 2008. 9: 1998 3. Ruprich-Robert, G, *et al.*,Genetics, 2002. 161: 1089 4. Espagne, E, *et al.*, Genome Biol, 2008. 9: R77

357. Biosynthesis of the histone deacetylase inhibitor depudecin from *Alternaria brassicicola*.
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The cyclic peptide HC-toxin, produced by the maize pathogen *Cochliobolus carbonum*, is an essential determinant of virulence. HC-toxin is an inhibitor of histone deacetylases (HDACs) in mammals and plants. *Alternaria brassicicola*, a pathogen of several Brassica species including *Arabidopsis thaliana*, produces a secondary metabolite called depudecin. Depudecin, a small polyketide, is also an HDAC inhibitor. To test the hypothesis that depudecin is a virulence factor for *A. brassicicola*, we are identifying and disrupting the genes for depudecin biosynthesis. *A. brassicicola* has nine polyketide synthase (PKS) genes. Targeted disruption of PKS9, but not of any of the other PKS genes, led to loss of depudecin production. PKS9 is part of an apparent gene cluster containing two monooxygenases, a transporter of the Major Facilitator Superfamily, and a possible transcription factor. In order to elucidate the complete biosynthetic pathway of depudecin, we are characterizing the structure and function of each of these genes. Mutant strains of *A. brassicicola* are being tested for altered virulence on Arabidopsis and cabbage. Preliminary results suggest that depudecin does not play a significant role in virulence.

358. Aconitase AcoA of *Aspergillus nidulans*, regulation at the atomic level?
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We study the propionate-degradation pathway of the filamentous fungus *A. nidulans*. In contrast to mammals, which use the methylmalonyl-CoA pathway for degradation, several microorganisms degrade propionate *via* the methylcitrate cycle (1). One of the key enzymes of this cycle is the methylaconitase, which we have shown to be the main aconitase (AcoA) of the citric acid cycle, too. This enzyme bears an interesting regulatory feature: the fully reduced 4Fe-4S cluster of this enzyme shows a constant ratio of aconitase and methylaconitase activity, whereby the oxidized 3Fe-4S cluster only shows methylaconitase activity. Therefore, the ratio of both activities indicates the oxidative state of the enzyme AcoA. We tested wild type cultures of *A. nidulans*, grown on different carbon sources for both enzymes. The different ratios implies the relevance of this regulatory mechanism *in vivo*. 1.Tabuchi, T., and Hara, S. (1974) Production of 2-methylisocitric acid from n-Paraffins

359. Functional characterisation of a nonribosomal peptide synthetase from the opportunistic pathogen *Aspergillus fumigatus*
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At least fourteen nonribosomal peptide synthetase (NRPS) genes are encoded within the *Aspergillus fumigatus* genome. Limited information is available relating these genes to a specific peptide product (e.g. siderophores or mycotoxins). This work aims to elucidate the NRP product synthesised by NRPS11 *(Afu6g12050)*, a monomodular NRPS, which does not appear to have homologues in other fungal species. RT-PCR confirmed the expression of *NRPS11* in *A. fumigatus (?aku80)* and gene disruption was undertaken to generate a *NRPS11* mutant termed *?NRPS11*. *NRPS11* gene expression was absent in the mutant (RT- PCR) under a number of culture conditions. Phenotypic analysis has confirmed increased sensitivity of *?NRPS11* to H_2O_2 (>1 mM) compared to wild-type *A. fumigatus*. These results indicate a role for NRPS11 in protection against oxidative stress within *A. fumigatus*. Comparative RP-HPLC analysis identified a conidial-specific metabolite (peptide) (Rt= 15.9 min; lambda max at 220nm) synthesised by *A. fumigatus* wild-type. This metabolite was absent from *?NRPS11* conidia. Increased production of this metabolite was observed in conidial extracts cultured in 2 mM H_2O_2, indicating up-regulation in response to oxidative stress and providing a promising candidate for a NRPS11 peptide. Future work will characterise this molecule by LC-MS and NMR. This work will contribute to the understanding on the role of NRPS in an important human pathogen.

360. Analysis of Putative Serine Proteases in *Aspergillus oryzae*.

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Aspergillus oryzae is an important microorganism, widely used in food industry. According to its genome analysis, *A. oryzae* is estimated to have 134 kinds of genes coding for protease. The number of the genes is about 30 percent more than that from other *Aspergillus sp*, and the functions of almost genes are still unknown. The purpose of this work is to express the predicted protease genes, and to reveal those functions. *In silico* analysis of serine and cysteine protease assumed their localization and annotation. As a result, oryzin, aorsin (aorsin A), aorsin homolog (aorsin B) were predicted to be extracellular serine proteases. We constructed the high expression system with *A. oryzae* as a host strain for expression of these proteases, and confirmed that these recombinant proteases were in culture supernatant. We analyzed enzymatic characteristic of these purified proteases including optimum pH and temperature, pH and temperature stability, inhibitor effect, and substrate specificity. In conclusion, these analyses revealed that there were differences of characteristic between these proteases. This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

361. HdaA, a class 2 histone deacetylase of *Aspergillus fumigatus* affects growth and secondary metabolite production

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Histone deacetylases (HDACs) play an important role in regulation of gene expression through histone modifications. Here we show that *Aspergillus fumigatus* HDAC is required for normal growth and is involved in regulation of secondary metabolite production. The deletion of the *hdaA* gene increased the production of several unidentified secondary metabolites but did not affect virulence of the fungus in a murine model. These results suggest that *A. fumigatus* HdaA has conserved functions, but also that distinct differences do exist among filamentous fungi. [This work was supported in part by the Seoul R&BD program to I.L.]

362. DON mycotoxin biosynthesis by Fusarium species, a metabolomics perspective.

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Many Fusarium species are fungal plant pathogens causing disease on both cereal and non-cereal hosts. Infection of the wheat ear typically results in bleaching and a subsequent reduction in grain yield. A larger proportion of the harvested grain may be spoiled by trichothecene mycotoxins such as deoxynivalenol (DON). Much progress has been made in the elucidation of genes required for trichothecene production, most of which are clustered at a single locus in the Fusarium genome. This project seeks to describe the metabolic characteristics associated with DON biosynthesis. As part of a new UK metabolomics initiative, we examined a range of well characterised wild-type Fusarium laboratory strains and single-gene deletion mutants under controlled conditions *in vitro*. A 'triple-fingerprint' of analytical techniques were employed to analyse the metabolome, composed of 1H-NMR and electrospray mass-spectroscopy (+/- ESI-MS). Principal components analyses of spectra were able to resolve *Fusarium graminearum, F. culmorum, F. pseudograminearum* and *F. venenatum* isolates after growth in liquid medium. In addition, several single-gene deletion strains that are reduced in pathogenicity exhibited large shifts in primary metabolism. Future work will attempt to find correlations between observed metabolic trends and DON biosynthesis.

363. Effect of phoshoglucose isomerase deletion on celullase production in *Trichoderma reesei*.

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Trichoderma reseei (anamorph *Hypocrea jecorina*) stands out for its ability to degrade cellulose, hemicellulose and xylan. Majority of the cellulase genes are repressed by carbon catabolite repression and are induced by plant-derived polymers, such as cellulose, their degradation products, and by some oligosaccharides, as lactose and sophorose. RutC30 is a well-known hypercellulolytic mutant, known to have a truncated *cre1* gene, a mutation in the ER glucosidase II alpha subunit gene as well as a large deletion of 85 kb. We have generated a phosphoglucose isomerase (PGI) deletant of the strain RutC30 in order to block glycolysis and direct carbon flux to pentose phosphate pathway (PPP). The latter pathway is considered to be the main source of NADPH that is needed for many biosynthesis pathways. The *pgi1* mutant strains constructed were shown to lack PGI activity and to have an active PPP. These mutants were able to produce more cellulases than RutC30 in media with glucose as the carbon source. The same pattern was observed in media with glucose and fructose, where both glycolysis and PPP are active. However, in media with lactose and glucose (L+G) or with lactose and fructose (L+F), RutC30 produced more cellulases than the disruptants. And in media with L+F, RutC30 showed a 10-fold higher cellulose level as compared with media with L+G. The mutants were able to maintain cellulase production for a longer time during the cultivation as compared to RutC30.

364. Using Metabolomics to identify targets of G-protein signalling in *Stagonospora nodorum*.

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Signal transduction pathways enable all living organisms to sense the environment and react appropriately. Failure to do so will have dire consequences, including abnormal cell development resulting in disease and/or death. Understanding this process at the molecular level has been the subject of intense scrutiny in all biological systems. Recent advances have been made in understanding the upstream components of signalling. What are less clear though, are the identities of downstream genes and proteins controlled by the signalling pathways. Strains of the wheat pathogen *Stagonospora nodorum* have been created lacking Galpha, Gbeta or Ggamma proteins. These strains all exhibit abnormal development and defects in pathogenicity. Our laboratory has exploited the emerging technique of Metabolomics to identify the downstream genes and proteins regulated by G-protein signalling. This poster will present recent data highlighting method development of the GC-MS metabolite screening technique as well as preliminary metabolite profiles of each of the strains.

365. Identification of gene *carD*, encoding the aldehyde dehydrogenase responsible for neurosporaxanthin biosynthesis in *Fusarium fujikuroi*

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Fusarium fujikuroi, well known for its ability to produce gibberellins, is also a model for genetic and biochemical analysis of carotenoid biosynthesis. Its major carotenoid product is neurosporaxanthin (NX), an acidic apocarotenoid formerly discovered in *N. crassa*. NX is produced in *F. fujikuroi* through the activity of the enzymes encoded by genes *carRA* (cyclase and phytoene synthase), *carB* (phytoene desaturase), and *carT* (torulene cleaving oxygenase). The enzyme responsible for the last reaction of the pathway, the oxidation of the aldehyde group of beta apo 4´carotenal to yield NX, has not been described in this fungus. Based on our former results with *ylo-1* in *N. crassa*, we have cloned the *F. fujikuroi* gene *carD*, coding for an aldehyde dehydrogenase putatively responsible of this enzymatic reaction. Crude protein extracts from an *E. coli* strain expressing a *carD* cDNA version were able to convert a C30 apocarotenal into the corresponding apocarotenoic acid, confirming the expected enzymatic activity. In contrast to other *car* genes, RT-PCR analyses of *carD* mRNA levels showed a light- independent expression. However, the mRNA levels were increased in carotenoid overproducing mutants. Phenotypic effect of targeted *carD* disruption, currently in progress, will be reported.

366. Isolation of a new putative transcription factor involved in the regulation of cephalosporin biosynthesis in *Acremonium chrysogenum*

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Primary and secondary metabolism in filamentous fungi is usually controlled by a network of transcription factors that act as activators or repressors on gene expression. In order to isolate proteins that bind specifically to promoter sequences we have developed a reliable method using a Streptavidin MicroBeads kit and biotinylated DNA-Fragments combined with Q-TOF spectrometry. The filamentous fungus *Acremonium chrysogenum* is the main producer of cephalosprin C. The biosynthesis of this beta lactam antibiotic is catalyzed by at least seven enzymes, two of which are expandase / hydroxylase and acetyltransferase [1]. These proteins are encoded by the *cefEF* and *cefG* genes, whose expression is driven by a strong 939 bp promoter. Using the biotinylated *cefEF*/*cefG*-promoter DNA sequence a method development was performed for the isolation of DNA binding proteins. Using a Streptavidin MicroBeads kit following Q-TOF spectrometry we were able to isolate at least one promoter binding protein which is involved in the regulation of cephalosporin biosynthesis. [1] Schmitt EK, Hoff B, Kück U (2004) Adv Biochem Engin/Biotechnol 88: 1-43

367. 4-Dihydrotrisporin-dehydrogenase from *Mucor mucedo*, a novel C4-oxidizing enzyme for trisporic acid synthesis

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Zygomycetes use retinoid-like ß-carotene derivatives, the trisporoids, as signal for partner recognition and internal regulation events during sexual development. Synthesis of these compounds involves oxidation of a hydroxyl group at the C4 atom in the ionone ring of the molecule. In the (-) mating type, this reaction occurs during conversion of 4- dihydromethyl trisporate to methyltrisporate. The analogous reaction converts 4- dihydrotrisporin into trisporin. Until recently, it was not clear if these reactions are catalyzed by a single or by different enzymes. We identified a C4-oxidizing enzyme from *Mucor mucedo* that is clearly different from the already characterized 4-dihydromethyltrisporate-dehydrogenase (TDH). This novel 26 000 enzyme prefers 4-dihydrotrisporin over 4-dihydromethyltrisporate as substrate and was therefore named 4-dihydrotrisporin-dehydrogenase (TNDH). We identified a single copy gene (TSP2) encoding a 240 amino acid short-chain dehydrogenase. Gene expression depends on developmental stage in both mating types. At the enzyme level, activity is found exclusively in the (-) mating type. Renaturation of proteins in SDS gels reveals, however, the presence of the TSP2 gene product in both mating types. This indicates posttranslational regulation of the 4-dihydrotrisporin dehydrogenase in the (+) mating type of *Mucor mucedo*. J. Wetzel et al. (2009) Eukaryotic Cell, in press.

368. An attempt to identify products synthesised by NPSs in *Fusarium graminearum*

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Non-ribosomal peptide synthetases (NPSs) are large multi-modular enzyme-complexes that produce small peptides. *F. graminearum*, has 19 potential NPS genes. The NPS19 is encoded by a ~8.9 Kb gene, flanked by a putative transmembrane ABC- transporters located 500 bp upstream to the NPS19. Here we have generated deletion (dNSP19) and over expression (ONSP19) mutants of the NPS19 gene and a deletion mutant of the ABC transporter (dABC) by Xi cloning and Agrobacterium mediated transformation. In addition an over expression strain of NSP19 containing a his-tag was generated by USER Friendly cloning. Two approaches will be used to identify the peptide: 1) 6 biological replicates of the dNSP19, ONSP19, dABC and WT (PH1) are grown in liquid cultures for 5 days. Untargeted analyses are performed by pressurized liquid extraction and RP-UPLC-ESI+-qTOF-MS and chemometric data- processing to identify potential NRP19 small molecule products. 2) Extracts of mycelium and culture medium from liquid grown dNSP19, ONSP19, dABC and WT (PH1) are fractionized by HPLC and each fraction is then tested for bioactivity on human cell lines.

369. Carbon metabolism and *Cryptococcus neoformans* virulence.

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Cryptococcus neoformans is an important fungal pathogen of immunocompromised individuals, with a close relative - *C. gattii* - emerging as a serious threat for the immunocompetent. During active infection, *C. neoformans* migrates to the brain and persists in the cerebrospinal fluid (CSF). Since CSF is a nutrient-limited environment, we sought to understand fungal carbon utilization in CSF. Prior studies have established the role of phosphoenolpyruvate carboxykinase (*PCK1*, links 3-carbon utilization with gluconeogenesis) in virulence using a inhalational mouse model. We evaluated a *pck1delta* mutant using a rabbit CSF model of virulence and found no defect in virulence for this mutant. Isocitrate lyase (*ICL1*), a key enzyme in the glyoxylate cycle (2-carbon utilization), was also previously examined by gene deletion and shown to have no impact on virulence in either the mouse or rabbit models of cryptococcal disease. In order to evaluate the use of various carbon sources by *C. neoformans* in the CSF environment, we created a *pck1delta/icl1delta* double mutant to evaluate the impairment of both 2- and 3- carbon utilization in a rabbit model of cryptococcosis. Furthermore, a pyruvate kinase (*PYK1*) mutant was made to examine the importance of glycolysis on growth and virulence in vivo. These studies serve to determine the relative roles of different carbon metabolism pathways in the persistence of *C. neoformans* in the CSF environment of the host.

370. Functional analysis of *fusA*, the PKS gene for fusarin production in *Fusarium fujikuroi*

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Fusarium fujikuroi is a rice pathogen which produces secondary metabolites with biotechnological applications. These include terpenoids, such as carotenoids and gibberellins, and polyketides, such as bikaverins and fusarins. The latter are micotoxins that at high concentrations provide a yellowish pigmentation to the mycelium. Little information is available on the regulation of fusarin biosynthesis by this fungus. We have found variations in fusarin production depending on temperature, nitrogen availability and light. The effect of these environmental signals has been also investigated in mutant SG62, formerly described as a fusarin overproducer. Polyketide biosynthesis starts through the activity of multidomain enzymes termed type I polyketide synthase (PKS). This functional diversity of these enzymes leads to the synthesis a wide variety of compounds. PKS genes are abundant in *Fusarium*, as exemplified by the 15 genes found in the *F. graminearum* genome. They include a PKS gene responsible for fusarin biosynthesis, also identified in another *Fusarium* species. The high sequence conservation between *Fusarium* genomes has allowed us to clone the orthologous gene in *F. fujikuroi*, which we named *fusA*. RT-PCR analyses currently in progress on *fusA* mRNA levels will be reported for different regulatory conditions. These analyses are extended to strains altered in fusarin production, such as SG62 and *wcoA* (white collar) mutants.

371. Mechanisms of regulation of amino acid transport and metabolism in phytopathogenic fungi

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How pathogenic fungi fulfill their nutritional needs during the interaction with their hosts remains poorly documented. Our studies focus on the amino-acid triggered regulation mechanisms required for the development of plant parasites. The gene encoding the monomeric GTPase Rheb, known to activate the kinase TOR, was silenced by RNAi in *Botrytis cinerea*. The implication of Rheb in the control of amino acid acquisition was appreciated with yeast complementation tests and the analysis of amino acid uptake/metabolism by reversed chromatography using HPLC. Mutants deleted of the gene encoding the bZIP transcription factor MetR, whose orthologue in *S. cerevisiae* controls the expression of the genes encoding the sulfate reduction pathway, were generated in *Magnaporthe grisea*. The putative MetR target genes were identified by testing the trophic requirements of the deleted strains, quantifying the intracellular amino acids and sulfur compounds by reversed chromatography and using transcriptomic approaches. The comparative analysis of the results obtained with both fungi –the *B. cinerea MetR* and *M. grisea Rheb* mutant strains being currently generated- will allow a better understanding of the mechanisms which control the transport and the metabolism of amino acids and sulfur compounds.

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372. Absence of *gliG* disrupts gliotoxin biosynthesis in the human pathogen *Aspergillus fumigatus*.

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Biosynthesis of gliotoxin is directed by a multi-gene cluster in the opportunistic fungal pathogen, *Aspergillus fumigatus*. The nonribosomal peptide synthetase GliP, responsible for condensation of Phe and Ser precursor amino acids, and the cluster regulator, GliZ, have been characterised. Minimal functional cluster annotation is available. The gene *gliG*, also found in a gene cluster, is classified as a glutathione s-transferase by *in silico* analysis. Two overlapping constructs, each containing part of a marker gene (*ptrA*), with homology to *gliG* flanking regions, were used to disrupt the *gliG* gene in *A. fumigatus* (Delta-*aku80*). The generation of a *gliG* mutant was confirmed using Southern Blot analysis using a digoxigenin labelled probe specific for an *XbaI* digested fragment size of 2124 bp in the wild-type and 1668 bp in the *gliG* mutant. Absence of *gliG* expression in the mutant was confirmed by Northern analysis. Interestingly, *gliG* expression was only evident in wild-type cultures in the presence of exogenous gliotoxin. RP-HPLC-DAD analysis of extracts from *A. fumigatus* wild- type and Delta-*gliG* revealed that gliotoxin (Rt= 14.4 min) is absent from the mutant strain, strongly indicating that *gliG* is involved in gliotoxin biosynthesis. Intriguingly, an additional metabolite (Rt = 12.3 min) was present in mutant culture supernatants, which may represent a precursor of gliotoxin (m/z: 271 – 293.2 by LC-MS). Work is currently ongoing to identify this molecule. Complementation of *gliG* is also in progress. Ultimately our results will provide an insight into the mechanistic route for gliotoxin biosynthesis.

373. Oxidative stress response and riboflavin production in *Ashbya gossypii*

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Ashbya gossypii (Eremothecium gossypii) is a filamentous fungus belonging to the family of *Saccharomycetaceae*. It is a known overproducer of riboflavin (vitamin B2). *Eremothecium cymbalariae*, a close relative of *A. gossypii* shows some morphological distinctions and does not overproduce riboflavin. It is known that addition of cAMP to the medium inhibits riboflavin production in *A. gossypii*. An *A. gossypii* strain deleted for *SOK2*, which encodes a transcription factor that plays a regulatory role in the cyclic AMP (cAMP)-dependent protein kinase (PKA) signal transduction pathway, results in strains that shows no riboflavin overproduction and appears white. Strains producing less riboflavin show an increased sensitivity against H_2O_2. In *S. cerevisiae* Yap1 controls the expression of genes in response to oxidative stress. In our effort to understand the biological role of riboflavin overproduction we, therefore, analyzed the oxidative stress response of *A. gossypii*. We generated an *Agyap1* mutant strain, which was found to produce less riboflavin than the parental strain. The sensitivity of *Agyap1* against H_2O_2 and menadione was increased. Using *lacZ* as reporter we found that Yap1 regulates the expression of *RIB4*, which encodes a gene of the riboflavin biosynthesis pathway. Comparison of AgYap1 with other fungal Yap1 proteins, e.g. *S. cerevisiae Yap1*, showed that AgYap1 lacks the characteristic cysteine rich domains. Surprisingly, these domains were found in the *E. cymbalariae* Yap1. A Hybrid Ag/Sc-Yap1 protein with the C-terminal cysteine rich domains of ScYap1 could complement the *Scyap1* H_2O_2 sensitivity, while AgYap1 could not. In conclusion, *A. gossypii* harbours a unique *YAP1* gene, which links the oxidative stress response with riboflavin production.

374. Homologues of the Aspergillus *velvet* complex control penicillin biosynthesis and morphogenesis in *Penicillium chrysogenum*

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The filamentous fungus *P. chrysogenum* is the main industrial producer of the pharmaceutical relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators. Recently, the so called *velvet* complex was isolated and functionally identified in *A. nidulans*. The main components of this complex compromising the velvet protein, a velvet-like protein VelB and the methyltransferase LaeA regulate both secondary metabolism and conidiospore development. In a first attempt, we have unambiguously identified homologues of all three genes in *P. chrysogenum* and generated deletion strains for further functional characterization using a *Pcku70* deletion strain with an improved gene targeting efficiency. Using expression and HPLC analyses, we have determined that the proteins characterized control penicillin biosynthesis. Furthermore, microscopic investigations such as bimolecular fluorescence complementation together with results from array analyses have shown that all components are important regulators of cellular differentiation in *P. chrysogenum*. All these findings extend options for industrial strain improvement programs.

375. Impacts on the metabolism of *Cryphonectria parasitica* by the virulence- attenuating virus CHV1-EP713.

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Cryphonectria parasitica, the chestnut blight fungus, can be infected by mycoviruses of the family *Hypoviridae*. Previous studies have led to the hypothesis that the hypovirus-infected phenotype is partly due to metabolic changes. To investigate further, we have measured the metabolic rate and respiration of colonies grown on solid medium. These experiments confirmed historical observations that the metabolic rate steadily declines with age and differentiation of the mycelium. Hypovirus infection caused an increase in metabolic rate in the youngest mycelium, but a subsequent decline was also observed. By measuring both CO_2 production and O_2 consumption, we have also observed that changes occur in carbohydrate metabolism as a result of aging in both infected and uninfected mycelium, but the hypovirus affects the extent of these changes. Finally, we have used metabolic profiling to determine the changes in accumulation of metabolites in wild-type and hypovirus-infected mycelium. These results have supported the physiological measurements, with alterations noted for lipids and carbohydrates. Additionally, we observed an increase in the accumulation of spermidine in the presence of hypovirus. Polyamines have been implicated in antiviral responses of mammalian systems, therefore this may suggest a novel antiviral response mechanism in fungi.

376. The structure of the third adenylation domain from the *Neotyphodium lolii* SidN non-ribosomal peptide synthetase

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Non-ribosomal peptide synthetases (NRPSs) are large multifunctional proteins that synthesise a diverse range of bioactive compounds, many of which have been shown to serve as pathogenicity or virulence factors in fungi, in addition to other roles such as iron acquisition through siderophore production. The adenylation (A) domains of NRPSs are primarily responsible for substrate specificity and activation of the selected amino acid. This substrate specificity can be successfully predicted for bacterial NRPSs from the structure of an A-domain binding pocket and the nature of the residues which line the pocket. However, this approach fails for eukaryotic NRPSs and, until now, fungal NRPS A- domains have been intractable to structure determination. We have solved the structure of the third adenylation domain from the *Neotyphodium lolii* NRPS, SidN (involved in synthesising an extracellular siderophore) by X ray crystallography. The structure is made up of two highly-conserved subdomains with a conformation not seen before in NRPS adenylation domains. In addition, SidN-A3 has 15 specificity conferring residues which line the binding pocket compared to just 10 for bacterial A-domains and could be why bacterial NRPS A-domain models fail to predict the substrates for fungal enzymes. Further studies will lead to the development of a general model for amino acid binding and activation by fungal NRPS A-domains.

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377. Respiratory Complexes III and IV are not essential for the assembly/stability of Complex I in fungi.

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According to recent insights, respiratory chains are commonly organized in large supermolecular structures termed ‘respirasomes’. In mammals, the respirasome model has been used to explain why certain mutations in one respiratory complex can cause secondary defects in others: Mutations affecting the assembly of Complex III has strong repercussions on the assembly of Complex I. Pharmacological interventions and mutations affecting only the activity of Complex III but not its assembly, do not have this effect. It thus appears that physical presence of Complex III is necessary for the assembly and/or stability of Complex I in mammals. Using native electrophoresis, we show that in the absence of either Complexes III or IV, the fungus *Podospora anserina* contains normal levels of Complex I, principally assembled into hetero-dimeric supercomplexes of the form I_1- IV_1 or I_1-III_2 respectively. These results suggest redundancy between Complexes III and IV with regard to their role in the assembly/stability of Complex I. Strikingly, also in the combined absence of Complexes III and IV, *P. anserina* contains normal levels of Complex I, a fraction of which is assembled into homo-dimeric supercomplexes of the form I_2. Single and combined deficiencies in Complexes III and IV are viable because of the presence of an alternative oxidase (AOX) found in fungi but not in mammals. Our results unequivocally demonstrate that, in contrast to the situation in mammals, Complexes III and IV are not essential for the assembly/stability of Complex I in fungi, possibly due to fungal-specific features of the respiratory chain like the presence of AOX and Complex I dimerization.

378. Regulation of bikaverin production by sucrose in wild type and *carS* mutants of *Fusarium fujikuroi*.

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Fusarium fujikuroi, the causal agent of Bakanae of rice, produces many secondary metabolites with biotechnological interest. Among them, stand out gibberellins (GAs), growth promoting plant hormones, carotenoids with antioxidant and pro-vitamin A properties, and bikaverins (BK), with activity against protozoa and tumor cell lines. Their biosyntheses depend on nitrogen starvation, but other environmental signals play regulatory roles, such as light, pH or aeration. We are interested in the effect of carbon sources and signals such as phosphate and sulphate, on the production of these metabolites. The effect of these nutrients was analyzed upon mycelial transfer to nitrogen starvation conditions. Wild type strain showed a 5-fold increase of BK production when transferred to a sucrose solution instead of glucose. However, GA or carotenoid biosyntheses were not affected. BK induction was not achieved at transcription level, as shown by northern and RT-PCR analyses. Stimulatory effect of sucrose was not so high for SG1, a carotenoid-overproducing mutant (*carS* phenotype) formerly described as deregulated for BK production in glucose. Other *carS* mutants tested produced more BK than the wild type when transferred to a glucose solution, but not when transferred to sucrose. Transfer experiments were achieved in the absence of other salts; BK induction by sucrose was lowered by phosphorus and sulfur in the transfer solution.

379. Screening of Secondary Metabolites Important in Fungal Interactions: The Challenge of Data Mining

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Fungi produce a vast diversity of chemical compounds. Secondary metabolites are especially important in the establishment and survival of these organisms in the environment. The purpose of this research is to identify and monitor secondary metabolites involved in the interaction of *Fusarium verticillioides* and *Ustilago maydis* in maize (*Zea mays*). A first step to achieving this goal is to screen for metabolites (i.e. fusaric acid, ustilagic acid, etc.) produced during *in vitro* interactions. We explore different strategies for data analysis in aims to answer three main questions: 1) What compounds (known and unknown) are produced by *F. verticillioides* and *U. maydis* in vitro?; 2) Is any compound up or down regulated as result of species' interactions?; 3) Is there any compounds produced *de novo* or completely suppressed during fungal interactions?. Isolates of *F. verticillioides* (endophytic isolates) and *U. maydis* were grown separately and in combination in solid media. Co-inoculated cultures fully colonized the media at day ten, time point when agar plugs where extracted for metabolic analyses. An UPLC/TOF/MS (Ultra High Performance Liquid Chromatography/Time of Flight/Mass Spectrometry) instrument was used for data collection and data processing was done with the software MarkerLynks (Waters Corporation). *U. maydis* chromatograms where highly complex containing approximately 100 peaks while chromatograms of *F. verticillioides* had less than 40. Thousands of markers (accurate masses that may include fragments and abducts) were detected across samples making data interpretation extremely difficult. Compound identification was assessed through the creation of a local database and by searches in public domain databases. Principal Component Analysis (PCA), PLS-DA (Projection to Latent Structures-Discriminant Analysis) and semi-quantitative analysis of identified compounds were also undertaken demonstrating that integration of diverse strategies is mandatory to comprehensively understand complex metabolic data.

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380. The heme biosynthetic pathway in *Aspergillus niger*

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The incorporation of heme as a cofactor, is a putative limiting factor in the overproduction of heme-containing fungal peroxidases in *Aspergillus* species. Addition of hemin to growth medium has been reported to improve the production of peroxidase. However, hemin uptake and the effect of hemin addition on the transcriptional regulation of the heme-biosynthesis pathway genes have not been studied in *Aspergillus*. To gain more insight into the heme biosynthesis pathway, the genes encoding the eight different enzymes in the pathway were identified in the A. niger genome. Individual deletion of four genes in the pathway (*hemA, hemB, hemF* or *hemH*) showed that all four are essential. In contrast to the other deletion strains, growth of the ?*hemA* mutant could be restored by the supplementation of 50 µM 5'- aminolevulinic acid (ALA). Supplementation with hemin alone did not restore growth, but supplying limiting amounts of ALA together with hemin fully restored growth of the ?*hemA* strain and indicates either enhanced uptake of ALA in the presence of hemin, or uptake of hemin itself. ?*hemB*, ?*hemF* and ?*hemH* strains could only be propagated as heterokaryons. The ?*hemF* and ?*hemH* heterokaryons show auto-fluorescence upon exposure to UV-light due to the accumulation of specific porphyrins. Possible regulation at the transcriptional level of the four heme pathway genes mentioned above under various heme-limiting conditions might provide more information about the regulation of the pathway, and is currently in progress.

381. Role of the 4-phosphopantetheinyl transferase in biological control by *Trichoderma virens*.

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Trichoderma species are used as biocontrol agents against phytopathogenic fungi. In the antagonistic interaction, they use secondary metabolites, which can be synthesized via pathways involving non-ribosomal peptide synthases (NRPS) and polyketide synthases (PKS). These enzymes require the addition of a prosthetic group called panthotein, which is incorporated by a 4-phosphopantetheinyl transferase (PPTasa). To determine whether PPTasa mutation affected the production of metabolites synthesized through the NRPS and PKS pathway, we obtained mutants carrying a deletion and over-expressed versions of the PPTasa gene in *T. virens*. The null mutant shows a fluffy phenotype, albino spores and is affected in the production of siderophores and antibiotics. As expected, 12 and 14 mer peptaibols were absent in comparison with the wild type strain. Over-expression of the PPTasa showed no effect in colony morphology and secondary metabolite profile, as compared to the wild type strain. Finally, the null mutant and wild type were used in seed germination assays *in vitro*, using tomato seeds, both strains promoted growth of tomato seedlings. However the null mutant showed less effect than the wild type strain.

382. Pigment production in response to deficiency in DNA repair

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Metabolites play an important role in many aspects of the fungal metabolism. We have observed that mutations in the homologous recombination (HR) apparatus in *Aspergillus nidulans*, which affect its ability to repair DNA damage such as double- strand breaks, result in the production of reddish pigment(s) coloring the fungal mycelium and surrounding media. The amount of pigment produced in the individual mutants of HR seems to correspond to the importance of the mutation in DNA repair. Colored fungal metabolites are often polyketides, a diverse group of secondary metabolites that has enormous interest due to their broad range of bioactivities in humans. By determining which compounds are involved in the response, we hope to understand how this fungus defends itself against DNA damage or replication stress via secondary metabolites.

383. Bicarbonate metabolism is involved in sexual reproduction of the filamentous fungus *Sordaria macrospora*

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Carbonic anhydrases (CA) are ubiquitous enzymes, which catalyze the reversible hydration of carbon dioxide. CAs from human, prokaryotes, plants and fungi exhibit only a low level of sequence identity and can be divided into different classes. Whereas 16 isoforms of human CAs were investigated, fewer CAs are encoded by fungal genomes. The yeast *Saccharomyces cerevisiae* contains one CA gene, two CA genes have been studied in the basidiomycete *Cryptococcus neoformans*. Whilst all human isozymes belong to the alpha-class, the beta-class is comprised of proteins from prokaryotes, plants, algae and fungi. Many prokaryotes as well as some ascomycetes contain CA genes from two different classes. In this study, we are presenting results concerning the characterization of the three beta-CAs in the filamentous ascomycete *S. macrospora*, termed *cas1*, *cas2* and *cas3* (**c**arbonic **a**nhydrase of **S**ordaria). To investigate the role of CAs, we deleted each gene and performed localization experiments. It turned out that the CAS2-protein localizes to mitochondria whereas CAS1 and CAS3 are cytoplasmic enzymes. Moreover, we demonstrated that CAS2 is required for ascospore germination and vegetative growth in *S. macrospora*; Delta_cas1 and Delta_cas3 mutant strains as well as the Delta_cas1/3 double deletion strain exhibit a wild-type like phenotype. Interestingly, severe defects in fruiting body development were observed in a Delta_cas1/2 double mutant. Under ambient air conditions, Delta_cas1/2 is no longer able to form any perithecia. A detailed characterization of single and double knockout mutants will be presented.

384. Expression of laccases in two lignin-degrading basidiomycetes during growth on wood

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Fungi, plants, insects, and bacteria produce laccases that are multicopper enzymes oxidizing phenolic compounds. In the presence of charge-transfer mediators laccases oxidize also non-phenolic compounds and are thereby connected to the fungal decay of wood lignin polymer. In addition, fungal laccases participate e.g. in the formation of fruiting bodies, sporulation, and plant pathogenesis. Due to their low substrate specificity, fungal laccases are used in various biotechnological applications. *Dichomitus squalens* and *Phlebia radiata* are white rot basidiomycetes that efficiently colonize wood and degrade wood lignin and lignin-like compounds. For this they express a set of extracellular lignin-modifying enzymes: *P. radiata* secretes laccases and lignin and manganese peroxidases (LiPs and MnPs) while *D. squalens* secretes laccases and MnPs. We followed expression of four distinct laccase genes (*Pr-lac1, Pr-lac2, Ds-lac1, Ds- lac2*) from *D. squalens* and *P. radiata* during their growth on Norway spruce with real-time quantitative RT-PCR. Glyceraldehyde 3-phosphate dehydrogenase was used as constantly expressed endogenous control gene to normalize the quantification of cDNA. This is the first time when laccase genes from *D. squalens* are cloned and characterized and their expression is followed on wood, which is natural substrate for white rot fungi.

385. Recombinant production of laccases by the basidiomycete *Coprinopis cinerea*.

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Coprinopis cinerea has seventeen laccase genes that in liquid culture are not or only poorly expressed. Strain FA2222 has been found to be laccase-negative at the normal growth of 37°C and is therefore used in transformations of laccase genes. For overproduction, laccases genes are linked to the *gpdII* of *Agaricus bisporus* (Kilaru et al. 2006, AMB 71:200). Laccase genes from *C. cinerea* and other basidiomycetes are tested for overexpression in *C. cinerea*. Differences in amounts of enzyme activities are encountered and differences in enzymatic properties. Culture conditions (media, temperature, inoculum, aeration and others) are optimized for highest production of recombinant enzymes in the fermenter. Cellular structures of hyphal pellets during fermentation are analyzed to better understand the link the growth form with best yields in enzyme production.

386. Cloning of Asparaginase gene, *ahrA*, from *Aspergillus nidulans*, and determination of specific enzyme activity using colorimetric methods.

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L-Asparaginase is an aminohydrolase that catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. The *ahrA* asparaginase gene (on chromosome VIII) produces a monomeric subunit that has 2 catalytic sites and a single tetrameric interface homology.
Now, this gene was purified from previously transformed *E.coli*, and sequenced to verify the existence of *ahrA*. The plasmid DNA was used in a PCR reaction with new primers containing BamH1 and HindIII restriction endonuclease sites. The PCR product was ligated into an expression vector (pET-21a(+), Novagen) and was transformed into bacterial strain DH5alpha. A mini-prep and restriction digest was performed to verify the presence of the expected insert into the plasmid. The purified plasmid DNA was transformed into bacterial strain BL21(DE3) that was induced with IPTG to express the enzyme. A 6x His tag allowed for the purification on Ni chelating beads and cleavage from the beads using increasing concentrations of imidizole. The specific enzyme activity (the production of ammonia) was measured by a colorimetric assay using sodium pentacyanonitrosylferrate as the reactive agent. Asparaginase is used as part of the therapy and cure for childhood acute lymphoblastic leukemia (ALL). The success of this research may have medicinal significance. I am grateful to Graduate Women in Science and San Diego State University Research Foundation for funding and laboratory space.

Population and Evolutionary Genetics

387. Multiple horizontal gene transfer events and domain fusions have created novel regulatory and metabolic networks in the oomycete genome.
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Complex enzymes with multiple catalytic activities are hypothesized to have evolved from more primitive precursors. Analysis of the *Phytophthora sojae* genome revealed 273 novel multifunctional proteins that are largely conserved across *Phytophthora* and *Pythium* genomes. Each of these proteins contains combinations of protein motifs that are not present in bacterial, plant, animal, or fungal genomes. Only 11% of these proteins models are also found in the diatom genome and thus the majority of these proteins have formed after the split between diatoms and oomycetes. We postulated that the novel multifunctional proteins of oomycetes might have value as potential Rosetta Stones to identify interacting proteins of conserved metabolic and regulatory networks in eukaryotic genomes. However ortholog analysis of each domain within the multifunctional proteins using the reciprocal smallest distance algorithm against 39 sequenced bacterial and eukaryotic genomes identified only 25 candidate Rosetta Stone proteins. Since the majority of proteins are not Rosetta Stones, they may instead serve to identify novel metabolic and regulatory networks in oomycetes. Since multifunctional proteins in metabolic pathways must act cooperatively with other components of the pathway, we looked in detail at the phylogenetic origins of enzymes in the sulfate assimilation, lysine and serine biosynthetic pathways. Each of these pathways had one or more bifunctional enzymes. Phylogenetic analysis of the proteins in these pathways revealed multiple examples of horizontal transfer from both bacterial genomes and the photosynthetic endosymbiont in the ancestral genome of Stramenopila.

388. Intra-isolate genetic variation in arbuscular mycorrhizal fungi persists at the expression level.
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Arbuscular mycorrhizal fungi (AMF) reproduce via multinucleate spores in which a surprisingly large amount of genetic variation has been reported, not only between individual spores but also between nuclei within spores. However, it is not clear to what extend genetic polymorphism persists in the transcriptome. Here we show that intra- isolate genomic variation is expressed in a ribosomal gene (25S rDNA) and protein- coding gene (PLS). We estimate copy number of the 25S gene to be on average 31 copies within the AMF Glomus intraradices, which is lower than the total amount of alleles observed and the lowest thus far in the fungal kingdom. Furthermore, genetic variation within 25S rDNA is not structured by isolate or geographical origin. Allele frequencies of the protein-coding gene PLS in the AMF G. etunicatum transcriptome differ from allele frequencies on the genome level. We have discovered a frameshift mutation in one PLS allele, thereby confirming the presence of at least one pseudogene. Our findings bring new insights into the significance of intra-organismal genome diversity in AMF. Understanding the genetics of these ubiquitous fungi is relevant both in the light of fundamental questions on genome evolution and to the potential of AMF to enhance sustainable agriculture.

389. Deciphering the genetic basis of speciation of a fungal plant pathogen through comparative genomics
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We compare the genome sequence of the wheat pathogen *Mycosphaerella graminicola* to the genome of a closely related pathogen (here named S1) infecting wild grasses to decipher patterns of genome evolution during speciation. Previous coalescence studies show that these two pathogens diverged 11.000 years ago in the Fertile Crescent coinciding with wheat domestication. We sequenced the genome of S1 using Solexa sequencing to an average coverage of 40X. Paired end reads were assembled using both a de novo assembly approach and by reference assembly to the already sequenced genome of *M. graminicola*. The assembled genome of S1 covers ~85% of the non repetitive fraction of *M. graminicola* nuclear chromosomes. Several structural rearrangements have occurred affecting mainly the seven dispensable chromosomes of *M. graminicola*. ~7Mb bases of sequence in S1 could not be aligned to the *M. graminicola* genome representing DNA that has either been lost from *M. graminicola* or inserted in S1 after their divergence. The 43kb mitochondrial sequence of *M. graminicola* was used as reference for assembly of the S1 mitochondrial genome. We obtained a coverage of ~740X providing us with an opportunity to detect and analyze mutational derivates within the sequenced individual. Conflicting sites within the S1 mitochondrion supports previous findings of heteroplasmy in *M. graminicola*.

390. Regulatory subfunctionalization and neofunctionalization account for the preservation of the ancient and extended cutinase family in *Magnaporthe oryzae*.
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The cuticle is the first barrier for fungi that parasitize plants systematically or opportunistically [1]. The functional characterization of three members of the cutinase family [2,3] of the destructive rice blast fungus, *Magnaporthe oryzae* [4] and the over-representation of cutinases in the sequenced genomes of Pezizomycotina render this a suitable multigene family to study the evolution of functional divergence of duplicates in fungal pathogens [5]. The family of cutinases shows extreme sequence diversity and is divided in two ancient subfamilies which predate the split between the two major fungal phyla, Ascomycota and Basidiomycota [1]. We discuss factors affecting the gene family size of the cutinase families between five Ascomycetes: the phytopathogens *M. oryzae*, *Fusarium graminearum* and *Botrytis cinerea*; and the model *Neurospora crassa* and *Aspergillus nidulans*. The average ratio of cutinase gene gain to loss is 2:3, with the exception of *M. oryzae* and *N. crassa*, which exhibit extreme family expansion and contraction, respectively [1]. The regulatory subfunctionalization and neofunctionalization of most *M. oryzae* cutinase gene pairs provide the first justification for the retention of paralogs after duplication and for gene redundancy in the genomes of fungal pathogens [5].
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391. Genetics and genomics of the chytrid pathogen of amphibia, *Batrachochytrium dendrobatidis*
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Chytridiomycosis is a potentially fatal, intracellular cutaneous disease infecting amphibians. It is caused by the fungus *Batrachochytrium dendrobatidis*, the first chytrid known to infect vertebrates. The disease was first described in 1998 and the responsible organism first isolated and identified in 1999. There is a world-wide pandemic of this fungus, leading to mass mortality, population declines and extinction of species of frogs. The fungus is widespread but multi-locus sequence studies indicate only limited genetic polymorphism, suggesting that chytridiomycosis is a recently emerged disease. Genomic analysis indicates that the organism is diploid. The chytrid has been maintained for extended periods in culture without evidence of recombination or a sexual cycle. Comparative genomics of chytrid strains from North America, Australia and New Zealand, supports the hypothesis that the chytrid pandemic is due to a recently emerged, clonal pathogen. This comparison includes the complete genome sequences of JEL423 (Broad) and JAM081 (JGI). The originating clone was, we believe, a hybrid between two somewhat different strains. There is evidence from comparative genomics that the chytrid is performing genetic recombination involving either a meiotic cycle or a novel form of mitotic recombination.

392. Spanish Neurospora: characterization of wild types isolated from the Iberian peninsula and the Canary Islands.
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Neurospora is found on burned vegetation from the tropic and subtropical regions throughout the world and through temperate regions of western North America and Europe. During the summers of 2006 and 2007 the Northwestern corner of the Iberian peninsula (Galicia, Spain) and the Canary Islands (North West of Africa) suffered unusually frequent wild fires that allowed us to sample the Neurospora populations in these regions. For each sample we amplified and sequenced three unlinked genetic markers corresponding to noncoding loci flanking microsatellites (TMI, DMG and TML). In addition we measured the amount of carotenoids in mycelia grown in the dark or after one day of light exposure. We isolated 131 new wild type strains of Neurospora: 73 from Galicia and nearby regions, and 68 from two islands of the Canary Islands archipelago (Santa Cruz de Tenerife and Gran Canarias). Initial comparisons with DNA sequences from selected Neurospora species allowed the assignment of each wild type to the corresponding phylogenetic group. Most sequences from the Iberian peninsula strains clustered with sequences from *N. discreta* while most sequences from the Canary Islands strains clustered with sequences from *N. crassa.* In addition, several sequences from both locations clustered with sequences from *N. tetrasperma* or *N. sitophila.* Our results will help to complete a map of the distribution of Neurospora in nature.

393. Mating-type chromosome evolution in the filamentous ascomycete *Neurospora tetrasperma*
Hanna Johannesson, Audrius Menkis and David Jacobson

We combined gene divergence data, classical genetics and phylogenetics to study the evolution of the mating-type chromosome in the filamentous ascomycete *Neurospora tetrasperma*. In this species, a large non-recombining region of the mating-type chromosome is associated with a unique fungal life cycle (pseudohomothallism) where self-fertility is enforced by maintenance of a constant state of heterokaryosis. Sequence divergence between alleles of 35 genes from the two single mating-type component strains (i.e. the homokaryotic *mat A* or *mat a*-strains), derived from one *N. tetrasperma* heterokaryon (*mat A* + *mat a*), was analyzed. By this approach we were able to identify the boundaries and size of the non-recombining region, and reveal insight into the history of recombination cessation. The non- recombining region covers almost 7 Mbp, over 75% of the chromosome. Furthermore, we found indications that the evolution of the mating-type chromosome in this lineage involved two successive events: two "evolutionary strata", highlighting shared features between the sex chromosomes found in the animal and plant kingdoms and the fungal mating-type chromosome, despite fungi having no separate sexes. Finally, in spite of an early origin of the first "evolutionary stratum", genealogies of 26 genes on the *mat*- chromosome from strains belonging to 9 reproductively isolated *N. tetrasperma* lineages indicate independent initiations and history of suppressed recombination in different phylogenetic lineages of the morphospecies.

394. Going HOG1 Wild at the BAR1: a Tale of Auto-eroticism in the budding yeast
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Organisms have evolved a wide variety of mating behaviors to ensure successful reproduction. The molecular details behind these strategies reveal evolutionary principles by which fitness through sexual reproduction is optimized. The budding yeast, *Sacchromyces cerevisiae*, has two mating types, **a** and alpha. Cells initiate mating by sensing pheromone produced by the opposite mating type. The mating type locus expresses transcriptional repressors and activators that regulate many sex-determining genes including those encoding pheromones and their receptors. We have found that a fraction of **a** cells produce alpha factor, the pheromone that their partners would normally make. At high densities, **a** cells that contain two mutations respond to this stimulation by arresting and forming mating protrusions. The mutations remove Bar1, the **a**-cell specific protease that degrades alpha factor, and Hog1 the MAP kinase involved in responding to increases in external osmolarity. This pathway and the pheromone response share a common MAP kinase kinase (Ste11) and in different circumstances the two pathways can either inhibit or reinforce each other. Even in strains that contain Bar1 and Hog1, some **a** cells express alpha factor. We speculate that this auto-erotic stimulation induces cells to produce a factor making them more attractive to alpha cells in the vicinity.

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395. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Far East of Russia.

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A multilocus genotyping (MLGT) assay revealed significant differences in the geographic distribution of 3ADON and 15ADON chemotypes of *F. graminearum* in Europe. While 93.5% of the isolates in southern Russia (N = 43 of 46) possessed the 15ADON chemotype, isolates in Finland and north-western Russia (N = 40) were exclusively 3ADON-producers during the years 1986-2006. In north-western Russia *F. graminearum* was not found until in 2003. All 27 *F. culmorum* isolates possessed the 3ADON chemotype, whereas all six isolates of *F. cerealis* possessed the NIV chemotype. In the Russian Far East (114 isolates) the 3ADON chemotype frequency increased between the years 1998-2006, and could reflect a shift in trichothecene chemotype composition similar to that observed within North America. However, additional sampling is required to confirm this observation. In addition, MLGT and phylogenetic analyses of multilocus DNA sequence data identified ten isolates from the Far East of Russia as a novel species, *F. ussurianum*, which together with *F. vorosii* and *F. asiaticum*, form a newly discovered Asian clade within the *F. graminearum* species complex. Pathogenicity to wheat and trichothecene toxin potential of the new species was also determined.

396. Evidence that the Vancouver Island Cryptococcus gattii outbreak has expanded into the United States Pacific NW

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Cryptococcus neoformans and *Cryptococcus gattii* are common fungal mammalian pathogens. *C. neoformans* is more prevalent, associated with pigeons in nature and a frequent cause of meningitis in immunocompromised patients, whereas *C. gattii* is geographically restricted to tropical and subtropical regions, associated with trees and usually infects immunocompetent individuals. Since 1999, an outbreak of *C. gattii* on Vancouver Island, British Columbia, has become endemic, caused numerous human and veterinary infections, and spread to mainland British Columbia. The outbreak isolates of C. gattii were characterized as molecular type VGIIa/major or VGIIb/minor. Beginning in 2006, human and veterinary cases have emerged in Washington State and Oregon. Using high-resolution multilocus sequence typing at a minimum of eight unlinked loci, we determined that most of these strains were VGIIa/major or VGIIb/minor, which provides direct evidence for the emergent spread of *C. gattii* from Vancouver Island to the Pacific Northwest of the United States. In addition, five isolates unique to Oregon and related to the VGIIa/major genotype form a novel cluster, which we have termed VGIIc. In addition, highly variable non-coding regions are under examination to further detect genomic differences among the VGIIa/major outbreak isolates. Continued analysis of veterinary, human, and environmental isolates from the region is ongoing, and the *C. gattii* Working Group of the Pacific Northwest has been established as a multidisciplinary effort to study the emergence. This unusual outbreak in a temperate climate raises concern about further expansion in the region and illustrates how microbial pathogens emerge in novel geographic locales.

397. Ancient isolation and recent migration patterns of the exotic sudden oak death pathogen *Phytophthora ramorum*

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In its known range, *P. ramorum* occurs as three distinct clonal lineages. We inferred the evolutionary history of *P. ramorum* from nuclear sequence data using coalescent-based approaches. The three lineages diverged for at least 11% of their history, on the order of 165,000 to 500,000 years ago. There was evidence for historical recombination between the lineages, indicating that the ancestors these lineages were sexually reproducing. Divergence of the three clonal lineages supports a scenario in which the three lineages originated from different geographic locations that were sufficiently isolated from each other to allow independent evolution prior to introduction to North America and Europe. It is thus probable that the emergence of *P. ramorum* in North America and Europe was the result of three independent migration events. Recent migration patterns of this pathogen in US nurseries were investigated using microsatellite analysis. Two distinct migration pathways, one containing isolates from Connecticut, Oregon, and Washington and the other isolates from California and the remaining states were identified. Together, these data suggest that migration, rapid mutation, and genetic drift all play a role in structuring the genetic diversity of contemporary *P. ramorum* populations.

398. Rapid expansion the emerging fungal disease chytridiomycosis into declining and healthy amphibian populations

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The fungal disease chytridiomycosis, caused by *Batrachochytrium dendrobatidis*, is enigmatic because it occurs in both healthy and declining amphibian populations. This distribution has fueled debate concerning whether, in sites where it has recently been found, the pathogen was introduced or is endemic. In this study, we addressed the molecular population genetics of a global collection of fungal strains from both declining and healthy populations using DNA sequence variation at 17 loci. The pathogen has extremely low DNA polymorphism and an excess of heterozygosity at multiple loci, consistent with a primarily clonal mode of reproduction. Nonetheless, a high diversity of multilocus diploid genotypes was observed, some of which we hypothesize could be explained by loss of heterozygosity through mitotic recombination. None of the geographic or host populations possessed the genetic signatures of a source population, though strains from temperate North America and Europe had slightly higher diversity than tropical populations. One strain isolated from a bullfrog contained as much genetic diversity as the entire global sample. These data are largely consistent with the fungus as a novel pathogen undergoing a rapid and recent range expansion.

399. The genotypic diversity of Phytophthora infestans in China shows a strong correlation with the region of origin

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We performed a comprehensive survey of *P.infestans* isolates in six important potato growing provinces in China. In 2006 and 2007, 119 isolates from potato and tomato were sampled and characterized. The isolates were genotyped using nuclear SSR markers and mitochondrial DNA markers. The mating type was determined using tester isolates. Based on the genotypic analysis a selection of isolates was tested for virulence on potato R-gene differentials. In two of the six regions both the A1 and A2 mating type were found, but genetic analysis did not indicate any sexual reproduction. In the other regions only the A1 mating type was found. The occurrence of the three mitochondrial haplotypes found (Ia, IIa, IIb), strongly correlated with the origin of the isolate. With the eight SSR markers a total of 40 genotypes were identified. Only two genotypes were present in more than one province while 38 genotypes were specific for their region of origin. The virulence of isolates on the potato differential set was identical within the same genotype, but varied strongly among isolates originating from different regions.

400. Evolution of a secondary metabolite biosynthetic gene cluster in *Fusarium* by gene relocation

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Trichothecenes mycotoxins are produced by some plant pathogenic species of *Fusarium* and can contribute to virulence on some plants. Previous analyses of *Fusarium graminearum* and *F. sporotrichioides* demonstrated that trichothecene biosynthetic enzymes are encoded at three loci: the single-gene *TRI101* locus; the two-gene *TRI1/TRI16* locus; and the 12-gene, core *TRI* cluster. Here, sequence analysis revealed that *TRI1* and *TRI101*, but not *TRI16*, are located in the core cluster in *F. equiseti* and *F. scirpi*. Examination of genome sequences of two distantly related, trichothecene-nonproducing species of *Fusarium* revealed remnants of *TRI101* in the same genetic environment as the intact *TRI101* in *F. graminearum* and *F. sporotrichioides*. This suggests that *TRI101* was present at this location, rather than in the core cluster, prior to divergence of trichothecene-producing and nonproducing species. Additional analyses of 16 trichothecene-producing Fusaria revealed that species phylogenies inferred from sequences of primary metabolic and core *TRI* cluster genes were correlated with genomic locations of *TRI1* in the species but not with phylogenies inferred from *TRI1* sequences. In addition, phylogenies inferred from *TRI16* sequences were highly correlated with those of *TRI1* regardless of whether the two genes were at the same locus. This pattern of congruent and incongruent phylogenies suggests that *TRI1* was located near *TRI16* but outside the core *TRI* cluster in the ancestral *Fusarium* and that *TRI1* moved into the cluster during the evolution of *F. equiseti* and *F. scirpi*. Thus, our results provide evidence that a filamentous fungal gene cluster can expand by rFebruary 18, 2009elocation of genes into the cluster from elsewhere in the same genome.

401. Assessing the performance of single-copy genes for recovering robust phylogenies

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Phylogenies involving nonmodel species are based on a few genes. Because gene trees are sometimes incongruent with species trees, the resulting phylogenies may not accurately reflect the evolutionary relationships among species. The increase in availability of genome sequences now provides large numbers of genes that could be used for building phylogenies. However, only a few genes can be sequenced for a wide range of species. Here we asked whether we can identify a few genes, among the single- copy genes common to most fungal genomes, that are sufficient for recovering accurate and well-supported phylogenies. Using 21 complete, publicly available fungal genomes with reliable protein predictions, 246 single-copy orthologous gene clusters were identified. We inferred the maximum likelihood trees using the individual orthologous sequences and constructed a reference tree from concatenated protein alignments. The topologies of the individual gene trees were compared to that of the reference tree using three different methods. The performance of individual genes in recovering the reference tree was highly variable. Two genes recovered exactly the same topology as the reference tree, and when concatenated provided high bootstrap values. The genes typically used for fungal phylogenies did not perform well, which suggests that current fungal phylogenies based on these genes may not accurately reflect the evolutionary relationships among species. Aguileta et al. Syst. Biol. 57(4):613–627, 2008

402. Spatial and temporal differences in the frequency of 3ADON-producing *Fusarium graminearum* populations in canadian wheat.

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Multilocus genotyping of 492 *Fusarium graminearum* from five Canadian provinces documented a 14-fold increase in 3ADON-producing *F. graminearum* between 1998 and 2004 in the three western Canadian provinces examined. Significant population structure associated with trichothecene chemotype differences was observed, and isolates from the 3ADON populations were found to accumulate significantly more trichothecene and had higher fecundity and growth rates, which may be driving their rapid spread. Expanded molecular surveillance based on 4,266 *F. graminearum* isolated from seven Canadian provinces between 2005 and 2007 demonstrated the trichothecene chemotype distribution in Canada was characterized by two longitudinal clines, one in eastern Canada and a second cline in western Canada. The frequency of 3ADON isolates continued to increase significantly in western Canada between 2005 and 2007. However, similar changes in chemotype frequency among isolates from eastern Canada were not observed. These data suggest a difference in selective pressure acting on FHB pathogens in eastern and western Canada or an uncoupling of the 3ADON chemotype from the trait or traits under selection in some eastern Canadian FHB populations.

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403. The origin and maintenance of genetic diversity within populations of the root pathogen *Armillaria mellea* in North America.

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Armillaria mellea (Basidiomycota, Physalacriaceae) *sensu stricto* is a common root pathogen of fruit crops, timber trees, and ornamentals worldwide. Phylogenetic analyses of nuclear rDNA and protein-coding loci support differentiation of four geographic groups: Asia, western N. America, eastern N. America and Europe. In N. America, western and eastern groups evolved from different ancestors, but their diversification from alternate ancestors has not been examined. To estimate origin, timing, and diversification from ancestral populations, 90 isolates were genotyped with tri- and tetra-repeat microsatellite loci, and haplotypes were constructed from nuclear and mitochondrial genes (Glyceraldehyde 3-phosphate dehydrogenase; GPD, ATP synthase subunit 6; ATP6). Lack of significant ATP6 sequence divergence among eastern N. America and European isolates, coupled with results of phylogenetic analyses of GPD, suggests a recent split inconsistent with continental connectivity. Eastern N. American populations show high levels of population admixture, although they are genetically structured across northern and southern regions. In western N. America, ATP6 sequence divergence shows two divergent haplotypes present in multiple populations, but GPD analyses do not support an ancient split. Introgression of both ATP6 haplotypes in western N. America, and the origin and extent of genetic diversity in eastern and western N. America appears to be inconsistent with expected biogeographic patterns of continental land masses.

404. Characterization of toxigenic Fusarium species in China

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The Fusarium genus is represented by a large number of evolutionary related but relatively diverse groups of organisms. Molecular research has shown that isolates that are morphologically similar represent different species. We characterized over 2500 single spore isolates collected from maize, wheat and barley from many different provinces in China using diagnostic primers for the different species, for their chemotype and by SSR markers. *F. asiaticum* was found to be the dominant species collected along the Yangtze River from wheat and barley samples. In the Northeast (Liaoning, Jilin, Heilongjiang) *Fusarium graminearum* sensu stricto was the dominant species on maize, but more to the West (Inner Mongolia and Henan province) *F. boothii* and *F. meridionale* were found. A clear distinction for the different species was found that related to geographic origin and host. In addition, barley isolates from the downstream valleys of the Yangtze River are mainly deoxynivalenol (DON) producers, while the vast majority of isolates collected in the mountainous provinces of Sichuan and Yunnan produce nivalenol (NIV). These data suggest a recent displacement of the Fusarium population in the valleys of the Yangtze River that is delayed by geographic barriers.

405. Analysis of naturally-occurring variation in fungal pathogenesis to plants.

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Naturally-occurring variation for most traits in all organisms is quantitative, i.e. variation is expressed on a continuous scale that cannot be categorized into separate groups. We are assessing *Cochliobolus heterostrophus*, causal agent of Southern Corn Leaf Blight disease, which has been used as a model for molecular genetic and genomic analyses of pathogenicity, as a model for studying the genetic basis of quantitative variation in pathogenesis. Some key questions must be addressed and developments are needed to study natural variation systematically in *C. heterostrophus*. Initial objectives of our project are to (1) assess the extent of natural variation in pathogenicity to maize of *C. heterostrophus*, (2) determine if that variation is heritable and, (3) develop an efficient system for processing populations of the fungus. This study will lay the groundwork for extending quantitative genetic analysis to the study of plant pathogen variation and evolution.

406. Phylogeny and historical biogeography of the true morels (*Morchella*).

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True morels (*Morchella*) are among the most charismatic of all edible macrofungi. Contributing to their allure is their short and sporadic fruiting season during the spring. Most species are distributed in temperate forests of the northern hemisphere, which suggests that they evolved in Laurasia. However, no detailed hypotheses have been proposed for their origin and diversification. Therefore, the present study was conducted to elucidate their species limits and biogeographic history by estimating their phylogeny using DNA sequence data from portions of four genes. These analyses resolved forty-two phylogenetically distinct species distributed among the following three lineages: *M. rufobrunnea*, and the *Elata* (black morels) and *Esculenta* (yellow morels) clades. The results revealed high continental endemism and provincialism in North America and Eurasia, suggesting long distance dispersal via ascospores may be rare, even on a continental scale. To develop a robust hypothesis of their biogeographic history, molecular divergence-time estimates were obtained to provide a temporal component to the phylogeny. Hypotheses were developed to help explain the timing of the origin and diversification of *Morchella*, and disjunctions between Old and New World species within the *Elata* and *Esculenta* clades.

407. Fungal diversity in deep-sea sediments - a whole new world?

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Deep-sea environment remains one of the most unknown research areas, holding great potential and interest for science. There have been many reports on the discovery of new organisms, life cycles and bio-resources from deep-sea environments. However, whereas the prokaryotic components of known deep-sea related organisms have been extensively described, very few studies have been focused on fungal communities associated with deep-sea environments. In this study, we examined the fungal diversity of 11 different deep-sea sediment samples (depth range between 200-10,000m) by amplifying the internal transcribed spacer (ITS) regions of rRNA genes with fungal- specific PCR primers. A total of 11 ITS libraries were constructed and 1056 clones were selected randomly. As a result of this study, some common fungal species in surface environments, such as *Penicillium*, *Aspergillus*, *Trichosporon* and *Candida* were identified. However, the majority of amplified ITS sequences were not associated with any known fungal sequences in the public database. Phylogenetic analyses suggested that some of these sequences could be included in the order of Chytridiomycota. Another interesting result was that one sequence was detected in all fungi-positive samples, despite the locality and depth difference. The most closely related fungal species of the sequence was *Metschnikowia colocasiae* (70% similarity). The results indicated that deep-sea sediments harbor diverse fungi, including new taxonomic groups that may be relevant to the early evolution of fungi and the discovery of new microbial metabolites.

408. Development of additional mitochondrial markers to assess evolution among the early diverging fungal lineages.

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Understanding evolution of the earliest fungal lineages remains one of the major challenges facing the Assembling the Fungal Tree of Life (AFTOL) project. In addition to the canonical AFTOL1 genes (nuclear-encoded SSU, ITS, LSU, RPB1, RPB2, EF1A; mitochondrial-encoded SSU, ATP6), AFTOL2 aims to develop new sources of comparative gene data to resolve deeper phylogenetic relationships. Mitochondrial rRNA markers may be most suitable for resolving intermediate levels of divergence (ex. Bruns and Szaro, 1992), however, a few protein-coding mitochondrial loci have been shown to resolve well-supported phylogenetic relationships in a study that included a small selection of fungi and other eukaryotes (Paquin et al., 1997). Since some mitochondrial markers appear to provide suitable resolution among early diverging fungi, this study aims to expand these data for a broader selection of taxa. Preliminary analysis of loci from the Fungal Mitochondrial Genome Project (Lang research group) shows that COX1, COX2, and COB contain convenient regions for primer design that targets a diverse array of chytrids and other early diverging fungi. Here we present new data and compare the support for basal fungal nodes obtained from mitochondrial versus nuclear-encoded markers.

409. Distribution of mating-type genes correlates with genetic recombination and aflatoxin- chemotype diversity in worldwide populations of *Aspergillus flavus* and *A. parasiticus*

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Aflatoxins are toxic polyketides produced by several *Aspergillus* species that contaminate food crops worldwide. *Aspergillus flavus* and *A. parasiticus* are the most common agents of aflatoxin contamination. The genes involved in aflatoxin biosynthesis are clustered and convert acetate and malonate to aflatoxins B_1, B_2, G_1, and G_2. We determined the frequency of the *MAT1-1* and *MAT1-2* mating-type genes in *A. parasiticus* and *A. flavus* sampled from single peanut fields in the United States (Georgia), Africa (Benin), Argentina, Australia, and India. Population samples for each species were clone corrected using multilocus sequence typing, which included two aflatoxin cluster regions (*hypE* and *aflW/aflX*), three non-cluster genes (*trpC*, *amdS* and a hypothetical protein encoding gene), and the *MAT* gene. Analysis of molecular sequence variation across 21 intergenic regions in the aflatoxin gene clusters of *A. flavus* and *A. parasiticus* revealed significant linkage disequilibrium (LD) organized into distinct blocks. To determine whether sexual reproduction gives rise to recombination blocks, we tested the null hypothesis of an equal number of *MAT1-1* and *MAT1-2* in populations sampled from each locality/species using a two-sided binomial test. For both *A. flavus* and *A. parasiticus*, when the number of *MAT1-1* and *MAT1-2* was significantly different in both corrected and uncorrected samples, there was more extensive LD in the cluster and isolates grouped into specific chemotypes, either the nonaflatoxigenic class in *A. flavus* or the B_1-dominant and G_1-dominant classes in *A. parasiticus*. In *A. flavus*, a 1:1 distribution of *MAT* genes reduces the frequency of nonaflatoxigenic strains and increases the resolution of LD blocks. In *A. parasiticus*, sexual reproduction and recombination reduces the frequency of B_1-dominant and G_1-dominant chemotypes, and isolate G_1/B_1 ratios show a continuous distribution in the population.

410. The *Schizophyllum commune thn1* gene serves as a trap for active mobile elements.

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The *thn1* gene of *S. commune* codes for an RGS protein that affects hyphal growth. Mutants with a corkscrew hyphal phenotype often spontaneously arise in laboratory strains and outgrow wild-type hyphae. Prior identification of the gene was aided by spontaneous insertion of the transposon *Scooter*. Here we hypothesized that *thn1* mutations frequently arise because an active *Scooter* close to the *thn1* locus regularly disrupts it. Sequences of mutant *thn1* alleles were investigated and the *S. commune* genome was searched for *Scooter* elements near *thn1*. Two elements with *Scooter* terminal inverted repeats (TIR) and a third with one TIR were located within ~500 kb of *thn1*. Analysis of seven additional *thn1* mutant alleles did not show *Scooter* as the disrupting mutation. However, two *thn1* alleles from other *S. commune* strains were disrupted at the same nucleotide position by mobile elements that are related to each other, but not to *Scooter*. One *S. commune* strain had been noted for *thn1* mutant hyphae for decades, while the second mutant arose recently in a different strain. A limited genome search found a DNA sequence identical to one of the insertions, but distant from the *thn1* locus. Thus the frequency of spontaneous *thn1* alleles may be a function of the ease of phenotype recognition and regular disruption of *thn1* by mobile DNA.

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411. Phylogeography of *Beauveria bassiana*, a complex of facultatively sexual entomopathogens.
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The anamorph *Beauveria bassiana* is a ubiquitous generalist entomopathogen. Previously treated as a single mitotically reproducing species, molecular phylogenetic evidence shows this taxon is made up of numerous morphologically cryptic lineages. One Asian species produces a *Cordyceps* teleomorph, contradicting the long-held view that *B. bassiana* is strictly asexual, although this example is an exception to the clonality observed elsewhere in the species range. To delineate phylogenetic species and their biogeographic ranges, an intraspecific phylogeny for a global sample of isolates was inferred from six nuclear intergenic regions totaling ~ 5500 bp. This phylogeny revealed four principal lineages- a basal clade whose haplotype diversity is greatest in North America and a paraphyletic sister lineage made up of three subclades, all of whose haplotype diversity is concentrated in Asia. All four lineages have undergone transoceanic dispersals to one or more continents relatively recently as inferred from the derived position of alleles representing these disjunct populations. A MAT1/2 PCR assay demonstrated that both mating types occur in all major and derived lineages of, thus the persistent asexuality of *Beauveria bassiana* can't be attributed to mating type losses in ancestral populations. However, limited population sampling in several species showed strong skew or fixation in mating types, thus uneven distributions of mating types within species may explain the strong bias toward mitotic reproduction in this species.

412. Elongation factor 2 phylogeny of *Olpidium* and its implications for early fungal evolution.
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Olpidium brassicae is one of the ~1000 fungal species that retained the unicellular, flagellated, aquatic habit that was present in the ancestor of all true fungi. Surprisingly, phylogenies from rDNA and RPB1 (James et al. 2006) placed *Olpidium* nearest terrestrial zygomycetes instead of among other unicellular water molds, albeit without bootstrap support. To challenge this result, we sequenced and analyzed the elongation factor 2 gene from zygomycetes and chytrids including two *Olpidium* species. Maximum likelihood analysis showed that the *Olpidium* species form a monophyletic clade with terrestrial entomophthoralean and harpellalean zygomycetes, but still without bootstrap support. Shimodaira-Hasegawa tests rejected the placement of *Olpidium* within the Chytridiomycota or Blastocladiomycota clades, however, placements basal to the Chytridiomycota or within the zygomycete clades were not significantly worse. *Olpidium* may represent one of the earliest diverging of all chytrid lines, or it could be the closest living aquatic relative of a terrestrial zygomycete lineage. Sequences from additional loci will help decide between these possibilities and either way, *Olpidium*'fs characters will provide basic information about features of early evolving fungi.

413. Sex and the *Trichoderma*: New perspectives for industrial strain improvement.
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The species *Trichoderma reesei* is industrially used for the production of cellulolytic and hemicellulolytic enzymes. All mutant strains used for biotechnological applications and research have been derived from one asexually propagating isolate, QM6a. The possibility to carry out sexual crossings with *T. reesei* QM6a would be highly desirable for basic research and industrial strain improvement. *Hypocrea jecorina*, a heterothallic species, was described to be the sexual form of *T. reesei*, but previous attempts of sexual crossings between *T. reesei* QM6a and *H. jecorina* strains were not successful. The aim of this study was to (re-)address the question if the industrial workhorse *T. reesei* QM6a is really an asexual clonal line. Analysis of the genome database revealed a *MAT1-2* mating type locus. We were able to isolate strains of the opposite *MAT1-1* mating type from a *H. jecorina* wild-type isolate. Upon mating with *T. reesei* QM6a those strains produced stromata and mature ascospores, thus enabling us for the first time to obtain sexual crossings with industrially used *T. reesei* strains. Both *MAT* loci from *H. jecorina* were characterized and we were able to convert the *MAT1-2* into the *MAT1-1* mating type in *H. jecorina* by targeted gene replacement and also successfully introduced the opposite mating type locus into *T. reesei*.

414. Variation and polymorphism of the mitochondrial genome of the conifer root rot fungus *Heterobasidion annosum s.s.*
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Heterobasidium annosum s.l. is a species complex that is pathogenic on a variety of conifers. In North America, there are two species with different host preference, pine (N. Am. P) or spruce/fir (N. Am S), and in Europe three species with preference for pine (Eur. P, *H. annosum s.s.*), spruce (Eur. S, *H. parviporum*) or fir (Eur. F, *H. abietinum*). In an earlier experiment with a hybrid from the N. Am. S and P types it was shown that the mitochondrial DNA (mtDNA) influences the fungal virulence on pine. The complete mitochondrial genomes of 24 individuals of the root rot fungus *H. annosum s.s.* (Eur. P) have been sequenced using the Solexa technology. The mtDNA of *H. annosum s.l.* appears to be substantially larger (114 kb) than reported in other fungal species. Sequence variation and polymorphism in mtDNA will be analysed for the 24 European *H. annosum s.s.* individuals. The presence of mitochondrial recombination will be addressed through analysis of linkage disequilibrium (LD) within the mitochondrial genome.

415. Systematics and population genetics of a phylogenetic species within the *Fusarium solani* species complex associated with human infections.
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The *Fusarium solani* species complex (FSSC) is a monophyletic group comprising dozens of phylogenetic and biological species, and represents the most common species complex associated with fusarial infections of mammals, particularly mycotic keratitis. Previous work found that approximately 75% of known FSSC isolates from human infections belonged to four phylogenetic groups (Zhang *et al*., J. Clin. Microbiol. 44:2186-2190. 2006). In the 2005-06 outbreaks of fusarial keratitis associated with a particular brand of contact lens solution, a large majority of isolates from infected corneas were members of one of these groups, previously termed Group 2. We used multilocus sequence data from portions of four genes and six microsatellite-harboring markers to investigate species boundaries and population structure associated with Group 2, including both clinical and environmental isolates. Phylogenetic analyses confirmed previous indications that FSSC Group 2 is a monophyletic group. PCR analysis indicated the presence of both MAT1 idiomorphs within the species, with no apparent phylogenetic clustering of idiomorphs, suggesting that there is an active, but at this time still cryptic sexual stage. Intraspecific analyses for population structure and recombination will be presented.

416. Genetic heterogeneity of iron transport gene *FET3* in *Debaryomyces hansenii* strains

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Phenotypic differences among strains of *Debaryomyces hansenii*, a marine yeast with significant biotechnological applications, may be an indication of genetic heterogeneity. However, current studies lack knowledge on the extent of the genetic divergence among strains and its possible role on a functional level. Genetic variations between *D. hansenii* strains CBS767^{T} and J26 were compared using DNA sequences of 18S rDNA and *FET3*, an important gene associated with iron transport and riboflavin production. We also report heterogeneity of the *FET3* gene in other strains of *D. hansenii* as compared to CBS767^{T} and J26 using RFLP analysis. These genetic differences may have a role to play in the responses of this organism to environmental stress.

417. Species boundaries of *Phytophthora capsici* and related taxa inferred from eleven nuclear and mitochondrial loci

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Phytophthora capsici causes extensive damage worldwide to a broad range of important agricultural plants. Localized field populations of *P. capsici* are reported to have considerable morphological and physiological diversity, including resistance to chemical controls. Previous studies provided evidence for subgroups within *P. capsici*, leading to descriptions of *P. mexicana* and *P. tropicalis*. We selected 75 isolates representing geographical, host and genetic diversity within *P. capsici* and related species, including *P. sp. glovera*, *P. tropicalis*, *P. siskiyouensis* and *P. mexicana*, to investigate species boundaries in this group. A phylogenetic analysis of these isolates using seven nuclear and four mitochondrial loci showed that they form a distinct monophyletic group, but there was no support for the status of *P. mexicana*. A novel, highly supported clade of *P. capsici* isolates from cacao in Brazil was also resolved, suggesting that these isolates constitute a new species. Two additional isolates also showed distant relationships to all other taxa, suggesting that they are single members of novel species. There was very strong support for the monophyly of *P. sp. glovera*, *P. tropicalis* and *P. siskiyouensis*. The results of analyses testing for historical recombination within the *P. mexicana/capsici* clade will also be presented. l

418. Genetic variability of mating genes and virulence factors of phytopathogenic fungi

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Ustilago maydis, the causal agent of corn smut disease, is a well established model organism to investigate highly specific pathogen-host interactions. In such an intimate coexistance, the emergence of new species depends on parameters related to virulence and resistance. In consequence, evolutionary studies of genes involved in those interactions are essential for the understanding of speciation processes. Smuts like *U. maydis* are characterized by a dimorphic life cycle with a haploid yeast and a dikaryotic parasitic stage linked by mating. After penetration the fungus proliferates within the host and finally diploid teliospores are produced. During this biotrophic phase many genes are crucial for the establishment of the interaction. In 2006, Kaemper and coworkers identified 12 gene clusters of secreted proteins with decisive functions in the infection process. Ongoing functional genetic approaches focus on those „biotrophy islands“ unveiling potential determinants of host specificity and virulence. Due to the direct interaction of parasites and hosts, speciation is linked to events of cospeciation, host shifts or host jumps. Therefore, we hypothesize that virulence factors are key players in speciation processes of the Ustilaginaceae. To test this, we performed a comparative population genetic approach investigating mutation rates of mating genes, potential virulence factors and housekeeping genes. Interestingly, mating genes show a similiar conservation than housekeeping genes indicating a low evolutionary rate. In contrast, virulence factors are highly variable proposing balancing selection on „biotrophy islands“. Based on our dataset, we will discuss the contribution of different genes to speciation processes.

419. Laccase and other multi-copper oxidase genes in Agaricomycotina

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Laccases as redox enzymes oxidize various phenolic compounds. Fungal laccases are thought to play roles in substrate degradation, in developmental processes and in stress reactions. Genomes of higher basidiomycetes offered the possibility to detect laccase genes by in silico methods. Surprisingly, higher basidiomycetes can have large families of laccase genes – for example, *Coprinopsis cinerea* has 17 different ones. In addition to laccase genes, other species have genes for related multi-copper-enzymes with poorly or not defined properties. Here, I present data on evolution of the families of laccase and other multi-copper enzymes. From species comparison it appears that genes duplicated late in evolution. Analysis of alleles of laccase genes in different strains of *C. cinerea* suggest that at least some of the duplicated genes can be lost without any problem. Data on gene expression through the fungal life-cycle will be presented as well as data on protein identification. Transformants of laccase genes under the control of the *gpdII* of *Agaricus bisporus* are used to produce high amounts of enzyme for protein purification and characterisation. Proteins encoded by the different laccase genes of *C. cinerea* are found to differ in enzymatic properties.

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420. Functions and phylogeny of fungal lignin and wood-modifying enzymes

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The biotechnologically promising lignin-modifying enzymes (LMEs) are secretory heme peroxidases and laccase-type of phenol oxidases produced by lignin-degrading, white-rot in wood causing or litter-decomposing basidiomycetous fungi. The core of LMEs are multiple peroxidases (LIPs, MNPs and VPs) forming several subgroups among fungal class II peroxidases, which belong to the heme peroxidase protein superfamily. Evolutionary grouping of the LME peroxidases coincides with their functional role and substrate affinity. Other class II peroxidases are enzymatically less characterized and diverge from the LME-peroxidases. Gene intron-exon structures, however, reveal high variations within the peroxidase subgroups. In addition, divergence in peroxidase gene inheritance, either sexually or via horizontal gene transfer may not be ruled out between the subgroups. For basidiomycetous laccases, paralogs are seen within the multicopper oxidase superfamily. However, the functional differences for several laccases sensu stricto described from one fungal species are not so obvious as for the fungal peroxidases. For other enzymes such as hydrogen peroxide generating oxidases and oxalate decarboxylases, their functions and phylogeny are also discussed in connection to the LMEs. Concatenated sequence analysis may in part predict what type of degradation pattern a polypore or corticioid fungus may cause on wood.

421. Variation and polymorphism of the mitochondrial genome of the conifer root rot fungus *Heterobasidion annosum s.s.*

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Heterobasidium annosum s.l. is a species complex that is pathogenic on a variety of conifers. In North America, there are two species with different host preference, pine (N. Am. P) or spruce/fir (N. Am S), and in Europe three species with preference for pine (Eur. P, *H. annosum s.s.*), spruce (Eur. S, *H. parviporum*) or fir (Eur. F, *H. abietinum*) have been described. In an earlier experiment with a hybrid from the N. Am. S and P types it was shown that the mitochondrial DNA (mtDNA) influences the fungal virulence on pine. The complete mitochondrial genomes of 24 individuals of the root rot fungus *H. annosum s.s.* (Eur. P) have been sequenced using Solexa technology. The mtDNA of *H. annosum s.l.* appears to be substantially larger (114 kb) than reported in other fungal species. Divergence Sequence variation and polymorphism in mtDNA will be analysed for the 24 European *H. annosum s.s.* individuals. The presence of mitochondrial recombination will be addressed through analysis of linkage disequilibrium (LD) within the mitochondrial genome.

422. The development of genetics and genomics for analysis of quantitative traits in the model filamentous fungus, *Neurospora crassa.*

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Our goal is to develop and distribute a set of strains and tools that will facilitate the identification of genes responsible for quantifiable phenotypes in Neurospora crassa. Mapping genes by linkage to phenotype has often suffered from poor resolution. As a result many mapped quantitative trait loci (QTLs) have not resulted in the identification of the contributing gene. The generation of a densely recombined mapping population and the parallel mapping of RNA expression and organismal phenotype will allow us to overcome this problem. We have generated a population of related strains for linkage mapping by an advanced intercross between strains that represent the two most distantly related known clades of N. crassa. These strains will allow us to map quantifiable phenotypes by linkage with high resolution. It has been shown that most mutations in a gene will result in an altered expression level for that gene. We are therefore sequencing cDNAs from each individual in the mapping population as well as the parents. We are using the short read technology developed by Solexa (now Illumina), which will allow us to generate genotyping data and measure gene expression simultaneously. By connecting the variation we observe in gene expression to genes found in QTL by the traditional correlation of phenotype and genotype, we will be able to identify the contributing mutations.

423. "Out of Africa" origin of human pathogenic fungus *Cryptococcus neoformans* var. *grubii*.

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Cryptoccocus neoformans var. *grubii* (serotype A) is the most common cause of fungal meningoencephalitis, one of the most prevalent and deadliest fungal infections in humans, which has a particularly devastating effect on AIDS patients in sub- Saharan Africa. Although this pathogen is ubiquitous around the world, yeast population in southern sub-Saharan Africa is genetically different from the global population. Here we present evidence that African population of the pathogen has a unique ecological niche in endemic African trees. We demonstrated that this niche harbors the ancestral yeast population, which represent s the evolutionary hotbed and center of speciation of *C. neoformans* var. *grubii*. We also demonstrate that global population of this fungus originated from a single expansion of two strains from the ancestral population in Africa, which became associated with the pigeon guano and were spread around the world by migration of humans and pigeons.

424. Conflict between reproductive gene trees and species phylogeny of outcrossing members of *Neurospora*

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Heterothallic members of the genus *Neurospora* (Ascomycota) are either one of the two mating types *mat a* or *mat A*. In this study we estimated the phylogenetic relationships of the mating-type genes (*mat a-1, mat A-1, mat A-2, mat A- 3*) and the pheromone receptor genes (*Pre-1, Pre-2*) among ten heterothallic and one pseudohomothallic taxa of *Neurospora*. The resulting genealogies were compared to the *Neurospora* species phylogeny derived from non-coding sequences (four microsatellite-flanking regions) published by Dettman *et al.* 2003. The results showed that genealogies of *mat A-1, mat A-2* and *mat A-3* were congruent. Comparison between *mat a* and *mat A* gene phylogenies revealed a conflict wherein the placement of the pseudohomothallic *N. tetrasperma* was incongruent in the different phylogenetic trees. We hypothesize that the origin of this species, and its unique life cycle with constant heterokaryosis for mating-type, may account for this result. Furthermore, our results indicate incongruence between reproductive gene trees and the species tree. The placement of *N. crassa* subgroup C and Phylogenetic species differed between all *mat*-genes and the species tree. The reason for this is uncertain but could be explained by incomplete lineage sorting, horizontal gene transfer or a hybridization event.

425. Does toxigenic synergy explain the co-existence of two emergent populations of *Fusarium graminearum* in the Upper Midwest?

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Large-scale surveys of the diversity of *Fusarium graminearum* in the upper midwestern U.S. have led to the identification of three co-existing populations. Members of these three populations can be distinguished from each other by neutral genetic markers and population membership is correlated with the spectrum of trichothecene mycotoxins produced in response to infection. The upper midwestern (UMW)3ADON population and UMW15ADON population have recently invaded the U.S. from Canada and are in the process of displacing the native Midwestern (MW)15ADON population. The UMW3ADON and UMW15ADON populations are genetically related, but can be distinguished by their trichothecene type, by their level of diversity within populations and by their inferred reproductive strategy that determines genotypic diversity; the UMW3ADON population appears to be recombining (outcrossing) while the UMW15ADON population appears to be clonal (selfing or asexually reproducing). In greenhouse experiments, average trichothecene (deoxynivalenol or DON) levels on wheat are correlated with population membership with UMW3ADON > UMW15ADON > MW15ADON. In field experiments however, this simple pattern is not observed as the UMW3ADON and MW15ADON populations cause DON to accumulate at similar levels. Nevertheless, a mixture of 2/3 UMW3ADON and 1/3 UMW15ADON inoculum resulted on average in 30% more DON in harvested wheat. As UMW15ADON and UMW3ADON individuals usually co- occur at these proportions in the Upper Midwest, we speculate that their co-existence may be maintained by this synergistic effect.

426. Comparative studies of the secretome of fungus-growing ants.

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Leafcutter ants of the species *Acromyrmex echinatior* live in symbiosis with the fungus *Leucoagaricus gongylophorus*. The ants harvest fragments of leaves and carry them to the nest where they place the material on the fungal colony. The fungus secretes a wide array of proteins to degrade the leaves into nutrients that the ants can feed on. The focus of this study is to discover, characterize and compare the secreted proteins. In order to do so cDNA libraries are constructed from mRNA extracted from the fungus material. The most efficient technology to screen cDNA libraries selectively for secreted and membrane-bound proteins is the TAST (Transposon Assisted Signal Trapping) technology (Becker et al. 2004,Microbiol Methods 57, 123-133). The TAST screening will give a list of full length gene sequences encoding secreted proteins. The main part of the secretome will consist of biomass degrading enzymes, but also antimicrobial proteins will probably be in the secretome. To date only a few enzymes from the *Leucoagaricus* secretome have been identified. We expect to discover novel proteins and to gain a better understanding of the biodegrading pathways of *Leucoagaricus*. Ultimately, this work may identify enzymes that can be used in biomass conversion processes.

427. Environmental DNA combined with fluorescent *in situ* hybridisation reveals a missing link in the fungal tree of life.

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Chytridiomycete fungi are important microbial components of aquatic environments. However, due to a reliance on culture based analysis, the evolutionary complexity of the 'chytrids' has been largely underestimated. Environmental gene library analyses of aquatic environments has recently demonstrated a highly novel form of deep branching 'fungi', the environmental diversity and complexity of which indicate that these uncharacterized-organisms are an important component of aquatic ecosystems. Here, we use a combination of environmental PCR and fluorescent *in situ* hybridisation (FISH) to elucidate the complexity and evolutionary history of these novel 'fungi'. PCR amplification and DNA sequence analysis extending through the 18S, 5.8S and 28S rRNA encoding genes, allowed improved phylogenetic analysis using complex models. The development of FISH probes reveals our target group to be a picoeukaryote of 4-5 micrometers in diameter and ubiquitous within local freshwater sites. Cells were morphologically similar and were often visualised in high numbers within structures we suggest to be sporangia. Interestingly, cell wall staining reveals our target organisms to be lacking in the characteristic fungal cell wall, unlike known 'chytrid' groups. This, in combination with our phylogenetic data, suggests this highly diverse group may be a missing link in the fungal tree of life.

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428. Multidrug-resistant *Botrytis cinerea* populations in French and German vineyards originate by different mutations leading to upregulation of ABC and MFS transporters

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In French and German wine-growing regions, increasing populations of *B. cinerea* with multidrug resistance (MDR) against commonly used fungicides have been observed in the last 15 years. Three types of MDR strains with different resistance spectra were distinguished. MDR3 strains, with the highest resistance levels, were shown to result from natural crosses of MDR1xMDR2 strains. All MDR strains showed increased efflux of various fungicides. MDR1 phenotypes are caused by different activating mutations in the coding region of a transcription factor gene (*mrr1*), leading to overexpression of *BcatrB* encoding an ABC transporter. All MDR2 strains analysed so far had suffered an identical insertion of a novel retroelement in the promoter of a MFS transporter gene (*mfsM2*), resulting in overexpression of *mfsM2*. Disruption of either *BcatrB*, *mrr1*, or *mfsM2* resulted in complete loss of the MDR phenotypes. Field tests confirmed that mixtures of modern fungicides select for MDR phenotypes in *B. cinerea*. Our work documents for the first time the widespread occurrence and molecular basis of selection for MDR in an important plant pathogen, similar to MDR in medically important *Candida* spp.

429. SNPs of information: Inferring evolutionary history in *Coccidioides*.

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Coccidioides spp., the causative agent of Coccidioidomycosis, is a dimorphic fungus, with a saprobic hyphal phase and a pathogenic spherule phase. We have previously shown that there are two species of *Coccidioides*, *C. immitis* (found in California and Mexico) and *C. posadasii* (found in Arizona, Texas, Mexico and South America). Recently, 4 strains of *C. immitis* and 10 strains of *C. posadasii* have been sequenced. Using a set of high-quality SNPs in these genomes, we have investigated effective population size, comparative SNP rates across different genomic features, patterns of positive selection in coding regions, and levels of conservation among vaccine candidates. We see that *C. posadasii* has a 2-fold larger effective population compared to *C. immitis*, but that *C. immitis* has more genes undergoing positive selection. Additionally, we have identified a set of conserved potential vaccine candidates. Using these data, we can make further hypotheses about the evolutionary history of *Coccidioides spp.* and inform the development of vaccine candidates.

430. Comparative evolutionary histories of the fungal chitinase gene family reveal non-random size expansions and contractions due to adaptive natural selection

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Gene duplication and loss play an important role in the evolution of novel functions and for shaping an organism's gene content. Recently, it was suggested that stress-related genes frequently are exposed to duplications and losses, while growth-related genes show selection against change in copy number. The fungal chitinase gene family constitutes an interesting case study of gene duplication and loss, as their biological roles include growth and development as well as more stress-responsive functions. We used genome sequence data to analyze the size of the chitinase gene family in different fungal taxa, which range from 1 in *Batrachochytrium dendrobatidis* and *Schizosaccharomyces pombe* to 34 in *Hypocrea virens*, and to infer their phylogenetic relationships. We also employ a stochastic birth and death model to show that the fungal chitinase gene family indeed evolves non-randomly, and we identify eight fungal lineages where larger-than-expected expansions (Pezizomycotina, *H. jecorina, H. virens, H. atroviridis, Gibberella zeae, Uncinocarpus reesii, Emericella nidulans* and *Rhizopus oryzae*), and two contractions (*Coccidioides immitis* and *S. pombe*) potentially indicate the action of adaptive natural selection. Expansions of chitinases putatively involved in antagonistic fungal-fungal interactions are discussed in relation to the ecological role and lifestyle of different fungal taxa.

431. Characterization of mutations affecting sexual reproduction in *Colletotrichum gloeosporioides*

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In filamentous Ascomycetes, heterothallic mating is determined by the MAT locus where each member of a mating pair carries a different idiomorph (MAT1-2 or MAT1-1) and homothallic strains carry both idiomorphs. Mating in the genus *Colletotrichum* however does not adhere to this system since MAT1-2 has been found in both mating partners in several species and to date no MAT1-1 idiomorph has been identified. Previous reports based on classical genetic analysis used the terms pseudo or unbalanced heterothallism to describe this phenomenon but to date no analysis has been carried out at the molecular level. In order to study the mating process in *Colletotrichum* in more detail, mutagenesis by T-DNA insertion was carried out on a homothallic *C. gloeosporioides* isolate obtained from papaya and mutants were screened for loss of homothallism or phenotypes showing altered reproductive structures. A large proportion of the mutants lost the capacity to reproduce as homothallics. However when crossed with other non-homothallics, the capacity for sexual reproduction was recovered as would be expected if unbalanced heterothallism was occurring. These mutants and a mutant producing aggregated rather than dispersed perithecia are currently being characterized in order to identify the affected genes. Reseach supported by CONACyT, Mexico.

432. Survey of *Neurospora sitophila* specimens reveals new phylogenetic species

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Phylogenetic species recognition has been applied to all of the biological species present in the heterothallic *Neurospora* clade except for *N. sitophila*. During our previous phylogenetic work with *N. perkinsii*, *N. metzenbergii*, and *N. hispaniola*, eight *N. sitophila* isolates were also included. The *N. sitophila* isolates did not group closely to previously sequenced *N. sitophila* isolates in our phylogenetic analysis leading us to believe there is much diversity and likely several distinct phylogenetic species within the *N. sitophila* biological species complex. In order to test our hypothesis about phylogenetic species, we have taken 80 *N. sitophila* isolates collected in nature from around the world, sequenced four unlinked loci, and constructed a phylogenetic tree to investigate how many groups in *N. sitophila* are genetically isolated and whether they are new phylogenetic species.

433. Molecular analysis of PRP8 inteins in the Botrytis genus illustrates a complex evolutionary history.

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Inteins are rare protein elements that are removed post-translationally from a host protein. This is an autocatalytic splicing process necessary for the maturation of the host protein. In addition to the essential splicing domains, most inteins encode a homing endonuclease (HEG). The HEG enables the intein to spread through the gene pool of a species and potentially to also undergo horizontal transmission to other species. The HEG introduces a highly specific double-stranded DNA break, which triggers the host's recombinational repair system. During this repair process, the host copies the intein with some of its flanking regions into the previously unoccupied target site. The current model of intein evolution proposes a cycle involving intein invasion, degeneration, loss and re- invasion. We have investigated the evolution of the PRP8 intein within the Botrytis genus. The PRP8 gene sequences of 22 Botrytis species and several closely related genera were investigated. The PRP8 gene of 15 species was occupied by an intein, 6 species carried an empty target allele, and once species was polymorphic for the presence/absence of the intein. Analysis of the intein sequences, their flanking regions and the sequences of the empty target alleles suggest that Botrytis has acquired the intein by horizontal transmission. We found no evidence of intein degeneration or loss in this genus.

434. From population genetics to population genomics in wood decay fungi

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Traditionally, population genetic studies have been based on allelic variation at a limited number of loci. Most studies have utilised neutral or near neutral markers for inferring geographic structure or demographic differences that sometimes have been compared to life history traits. With a lower cost for and an increasing access of DNA sequence data whole genome sequences can be used for inferring population structure. In addition to giving a much stronger statistical support for the results, the approach opens up for scans for information of linkage disequilibrium, signs of selection, genetic mapping of functional traits, subspecies detection etc. This presentation will give examples of traditional population genetics work in decay fungi by using whole genome sequence data from several individuals within the *Heterobasidion annosum* species complex to show how population genomic work might develop to ask and answer questions on species phylogeny and on evolution of neutral and adaptive traits.

435. Population genomics in the forest pathogen *Heterobasidion annosum*.

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Heterobasdion annosum s.s., a pathogenic basidiomycete causing root and butt rot to conifers, is found across Europe. It is part of the *H. annosum s.l.* species complex, with members in both Europe and North America and host specificities towards different conifers. The pathogenic lifestyle as well as the possibility to obtain monokaryotic isolates makes this species interesting and suitable for population genomic analysis. Recent advances in sequencing technologies have enabled the move from population genetics to population genomics. We have sequenced the genomes of several individuals of *H. annosum s.s.* using the sequencing by synthesis Illumina/Solexa platform. We have used *de novo* assembly as well as mapping of sequencing reads to the genome of a closely related *H. annosum* species to reconstruct the genomes of the sequenced individuals. This allows us to describe the genomic variation between the individuals within the focal species. Single nucleotide polymorphism (SNP) mining in the Solexa reads provided more than 100 000 SNPs. On a whole genome scale, we address questions of population structure, linkage disequilibrium and recent selective events.

436. A comparative analysis of regulatory regions in the Fusarium trichothecene gene cluster

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The ability of Fusarium species to produce trichothecenes such as deoxynivalenol (DON) is determined by the co-expression of several genes belonging to a main biosynthetic cluster of 25 kb together with two other known unlinked genes. Activation of TRI biosynthetic genes is due to the binding of Tri6p, a Zn finger protein, probably interacting with Tri10p and other unidentified regulators. In order to analyse the regulation of TRI cluster genes in Fusarium type B trichothecene producers, we partially sequenced the TRI cluster of various Fusarium strains (n=35) from Luxembourg, and compared them with previously sequenced strains from other locations (USA, Africa, Europe, South America, Asia). We then applied bioinformatic methods based on genomic fingerprinting in order to identify other putative regulatory regions of the cluster. We aligned non-coding regions within the cluster showing that the regulatory region of trichothecene B producers suffices for discriminating chemotypes. We found that the positions and abundance of a known transcription factor binding site involved in Tri6p regulation are correlated (100%) to chemotype in the *Fusarium graminearum* species complex. Moreover, a new PCR-assay based on a regulatory region has been designed for the identification of 3-acetyl-DON producers for screening Luxembourg Fusarium population. Functional studies for validating the activity of the newly identified putative regulatory motifs in the TRI cluster are ongoing.

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437. Fungal insect competition and the secondary metabolism

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Filamentous fungi and saprophage insects are suspected to be competitors on decaying organic matter. Both organisms have equal requirements considering habitat and nutrition. Insect larvae negatively influence mould development (1), but filamentous fungi can be an important cause of mortality of insect larvae (2). These competitions in insect- mould interactions have largely been ignored. First investigations suggest a role of genes for fungal secondary metabolism (3). We ought to be investigate the function of moulds secondary metabolites as a chemical defence in insects-moulds-interactions as well as the influence of these competitors at trophic interaction between insects . Microarrays of secondary metabolism genes of *A. nidulans* are being used to identify fungal target genes up- or downregulated when interacting on festered matter with the antagonistic Drosophila larvae. Preliminary tests employing real time RT-PCR with RNA from *A. nidulans* confronted with *D. melanogaster* larvae indicates upregulation of the global regulator laeA, as well as aflR and sterigmatocystin biosynthesis genes. The consequence on evolutionary fitness of fungi and insects will be discussed.

(1) Rohlfs M (2005) Mycologia 97:996-1001 (2) Rohlfs M (2005) Frontiers in Zoology 2:2 (3) Rohlfs M, Albert M, Keller NP, Kempken F (2007) Biol Lett, doi:10.1098/rsbl.2007.0338

Host Pathogen Interactions

438. Cin1, a novel repeat protein secreted during the early stages of infection of apple by *Venturia inaequalis*.

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Venturia inaequalis is a hemibiotrophic ascomycete that causes apple scab. Germ tubes, from conidia or ascospores, penetrate the leaf or fruit surface directly via appressoria-like swellings; subsequently the hyphae divide laterally to form a stroma between the cuticle and the outer wall of the epidermal cells. Stromata can be induced by growing the fungus *in vitro* on cellophane discs. *cin1* (*c*ellophane- *in*duced-1), was identified from a differential cDNA library screen of mycelia grown on cellophane and liquid culture. Real-time quantitative PCR showed that *cin1* was up-regulated over a thousand fold in infected apple leaves compared with liquid culture. Cin1 has seven or eight imperfect repeats of 60-65 amino acids, depending on the isolate, is secreted, but has no similarity to sequences in publicly available databases. We have used RNAi to knockdown the expression of *cin1*, and these transformants are non-pathogenic. The phenotype of the knockdowns and the analysis of *cin1* expression using enhanced-yellow fluorescent protein fusions will be presented.

439. A novel family of secreted proteins in arbuscular mycorrhizal fungi

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Secretion of effector proteins that interfere with plant defence mechanisms is of major importance for bacterial, oomycete and fungal plant pathogens to establish disease. In particular for biotrophic fungi, involved into long-term relationships with their plant partner, a permanent suppression of the defence response is essential to fulfil their lifecycle within the host. This is especially true for arbuscular mycorrhizal (AM) fungi that maintain their biotrophic status during their whole life cycle. However, almost nothing is known about the AM fungal effectors that might be involved. To search for potential effector proteins from AM fungi, we generated several cDNA libraries of *Glomus intraradices* at different developmental stages of the symbiosis including pre- symbiotic mycelium, appressoria, arbuscule and extraradical mycelium. Screening for secreted proteins, using the yeast signal sequence trap method, we identified many so far unknown proteins from *G. intraradices* secreted either through a classical amino terminal secretion peptide or through a non-classical secretion mechanism. Among the isolated sequences, a novel family of fungal proteins with hydrophilic repeats and nuclear localisation signals was identified. Heterologous expression systems could show that the NLS are functional and that the proteins without signal peptide are targeted to the nucleus. We hypothesized that these proteins are fungal effectors that after entering the plant cytoplasm are targeted to the nucleus to manipulate the host cell program. To investigate the function of these putative effector proteins we have carried out Y2H analyses and the results will be presented.

440. Amino acid residues involved in fungal hydrophobin RolA and cutinae CutL1 interaction.

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When fungi grow on plant or insect surfaces coated with wax polyesters that protect against pathogens, the fungi generally form aerial hyphae to contact the surfaces. Aerial structures such as hyphae and conidiophores are coated with hydrophobins, which are surface-active proteins involved in adhesion to hydrophobic surfaces. When the industrial fungus *Aspergillus oryzae* is cultivated in a liquid medium containing a biodegradable polyester polybutylene succinate-coadipate (PBSA), a Type I hydrophobin RolA is largely secreted into the medium. Under these conditions, *A. oryzae* simultaneously produces the cutinase CutL1, which hydrolyzes PBSA. RolA attached to the hydrophobic surface of PBSA particles in the medium specifically recruits CutL1, resulting in stimulation of PBSA hydrolysis (1). In the present study, we studied amino acid residues involved in the RolA-CutL1 interaction by means of site-directed mutagenesis and chemical modification of CutL1 and RolA. Teflon particles were coated with RolA and its derivatives. Then the Teflon particles coated with RolA were incubated with CutL1 and its derivatives for recruitment. Recruited CutL1 was extracted from the centrifuged particles with SDS and quantitatively measured by SDS-PAGE analyses. We found that His32 of RolA and peripheral acidic amino acids of Cutl1 are required for the for RolA-CutL1 interaction. (1) Takahashi T. et al. Mol Microbiol. 57:1780 (2005)

441. A Zn(II)2Cys6-type transcription regulator embedded in the AM-toxin biosynthetic gene cluster negatively controls the toxin biosynthesis in the apple pathotype of *Alternaria alternata*.

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The apple pathotype of *Alternaria alternata* produces host-specific AM-toxin and causes Alternaria blotch of apple. Previously, we identified four *AMT* genes (*AMT1-AMT4*) involved in AM-toxin biosynthesis and found that these genes are encoded by small, supernumerary chromosomes of <1.8 Mb in the apple pathotype strains. In this study, we identified a BAC clone, AM-BAC-14 (117,849 bp), that contains the four *AMT* genes and 23 putative genes. Real-time quantitative PCR analysis of the transcript levels of the genes in AM-toxin producing and non-producing cultures showed that 14 genes, including four *AMT* genes, were upregulated (>5-fold) in toxin producing culture. These genes are encoded within the 70-kb region in AM-BAC-14, suggesting that this region corresponds to the AM-toxin biosynthetic gene cluster. We identified a gene, named *AMTR1*, which encodes a Zn(II)2Cys6-type transcription regulator, in this region. Unexpectedly, disruption of *AMTR1* in the wild-type strain IFO8984 markedly enhanced AM-toxin production. In contrast, overexpression of *AMTR1* in IFO8984 reduced AM-toxin production. We verified that the transcript levels of members of the putative *AMT* gene cluster were repressed in the *AMTR1*-overexpressing strains. These data indicate that *AMTR1* encodes a transcription regulator negatively controlling AM-toxin biosynthesis in the apple pathotype of *A. alternata*.

442. Withdrawn

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443. What makes a biotrophic fungus a plant-pathogen or a symbiont ? Insights from transportome analysis.

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Poplar trees are common used for production of pulp and consolidated wood products. More recently, woody plants are of great interest for carbon sequestration, biofuels production, and bioremediation. *Populus* is able to interact with biotrophic fungus, such as the symbiotic (*Laccaria bicolor*) and pathogenic (*Melampsora larici- populina*) basidiomycetes. During a plant-biotrophic fungus interaction, there is a fine-tuned metabolic association between partners. In pathogenic interaction, unilateral links predominate, whereas in symbiosis exchanges are bi-directional. Membrane transporters are thus key molecular players. Genomes of symbiotic (*L.bicolor*) and pathogenic (*M. larici-populina*) basidiomycetes interacting with *Populus* are now available. We take advantage of these genomic resources in order to identify genetic potential for membrane-transporters (transportome) and to provide insights into pathogenicity/symbiosis mechanisms. Results from comparative genomics and micro-arrays expression analysis will be presented.

444. Regulation of the isocitrate lyase encoding gene, acuD in the opportunistic human fungal pathogen Penicillium marneffei

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Infecton by the human pathogen Penicillium marneffei is initiated by the inhalation of conidia and eventually leads to a disseminated mycosis if untreated. The inhaled conidia are phagocytosed by host alveolar macrophages and, rather than being destroyed, proliferate intracellularly. Therefore the ability of P. marneffei to escape phagocyte killing and grow in this environment, which is low in available glucose and high in alternative carbon sources such as fatty acids, is an important factor in pathogenicity. Utilisation of alternative carbon sources, which are catabolized to acetyl-CoA requires flux through the glyoxylate cycle to generate intermediates of the tricarboxylic acid (TCA) cycle. The glyoxylate cycle is present in many fungi and bacteria but lacking is larger eukaryotes. In P. marneffei the first unique enzyme of the glyoxylate cycle, isocitrate lyase, is encoded by acuD. Expression of acuD is induced in response to macrophage engulfment in vitro, acetate as a carbon source and a shift to 37°C, with temperature-dependent expression over-riding glucose repression at 37°C. Identifying the temperature dependent repressor(s) of acuD is important for understanding the infection processes. Promoter deletion analysis of acuD has revealed a putative temperature-dependent repressor of acuD in the region -1300 to -687. This region of the promoter contains putative binding sites for both the developmental regulator AbaA and the fatty acid regulatory factors FarA/FarB, suggesting that developmental and carbon source-dependent regulation of acuD occurs through this one region. The P. marneffei farA and farB genes have been cloned and their role in the regulation of acuD in response to carbon source and temperature examined.

445. Proteins involved in attack and defence in Zygomycete-aphid interactions.

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Fungi within the order Entomophthorales (Zygomycota) are obligate biotrophic pathogens of insects. Initially, the fungi penetrate the cuticle of their host, where after they grow with a protoplast-like morphology within the host, slowly degrading and eventually killing it. We have performed a secretome study of field collected grain aphids (*Sitobion avenae*) in early stages of infection with entomophthoralean fungi using the transposon assisted signal trapping (TAST) technology (Becker et al. 2004, J Microbiol Methods 57, 123-133), thereby discovering a series of secreted enzymes of the pathogens and defense related proteins of the host. Now, selected proteins are being produced in an expression host for further studies. The conservation of the fungal secreted proteins in the species dominating the collected material, namely *Pandora neoaphidis*, *Entomophthora planchoniana* and *Conidiobolus obscurus*, and additional related fungi are under investigation. We anticipate that our work will shed light on this highly specialized group of fungi that has attracted substantial attention as potential bio-control agents.

446. Withdrawn

447. Cpkk1, a Mapkk of *Cryphonectria parasitica*, is necessary for virulence on chestnut trees

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In order to investigate the role of two MAPKK, Cpkk1 and Cpkk2, in *Cryphonectria parasitica*, we generated a number of mutant strains for each gene, which under the control of the cryparin promoter were overexpressed both as a wild type protein and with an extensive deletion in the catalytic domain. Furthermore, to cause specific silencing of each mRNA transcript, hairpin constructs of each of the two MAPKK were expressed. Initial screening of the transformed strains was conducted by the evaluation of mRNA expression levels with qRT-PCR. Specific silencing or overexpression was confirmed with both northern and western blot analysis. Selected *C. parasitica* strains with Cpkk1 or Cpkk2 both silenced and overexpressed were evaluated for certain biological properties including virulence on European Chestnut (*Castanea sativa*), competence in sexual mating, growth rate on different substrates, conidial sporulation and the resistance to cell wall degrading enzymes. Silencing of Cpkk1 (but not of Cpkk2), and the overexpression of a defective Cpkk1 correlated with marked reduced virulence on 3 year old chestnut trees with no statistically significant effect on growth rate in the various conditions tested.

448. Dothistromin biosynthesis in the pine-infecting fungus, *Dothistroma* spp.

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Dothistromin is a non host-specific toxin produced by several fungi including the pine needle pathogen *Dothistroma septosporum*. It is similar in structure to versicolorin B, an aflatoxin precursor, and is produced in culture and *in planta*. The red bands typically seen in *Dothistroma*-infected pine needles are principally due to accumulation of this toxin. Injection of purified dothistromin into needles led to development of typical disease symptoms, suggesting a major role in disease. However, studies with toxin-deficient mutants of *D. septosporum* showed that dothistromin is not required for pathogenicity. Mutant strains were able to colonise needles, trigger lesion formation and sporulate the same as dothistromin-producing strains. Dothistromin is unusual among fungal secondary metabolites in being produced at a very early stage of growth, rather than in late exponential or stationary phase. The early onset of dothistromin production, along with its broad-spectrum toxicity, led to our current hypothesis that dothistromin has a role in protection against other microorganisms in the needle environment. *In vitro* studies with needle-dwelling organisms support this. We further propose that dothistromin mainly functions to inhibit growth of other microorganisms within the needle, such as endophytes and latent pathogens, and will present our reasoning for this speculation.

449. Identifying regulators of apothecium development in *Sclerotinia sclerotiorum* by *Agrobacterium*-mediated transformation

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Using *Agrobacterium tumefaciens*-Mediated Transformation (AT) for insertional mutagenesis of *Sclerotinia sclerotiorum*, we have generated and screened 1,045 transformants for apothecium disc development defect phenotypes. Twenty-two strains have been identified that fall broadly within four phenotypic classes: (1) stunted stipes with no disc development, (2) convoluted disc morphology, (3) non-expanding discs and (4) miniature apothecia. Flanking nucleotides sequences have been recovered for one non-expanding disc mutant (AT-90) and one miniature apothecium mutant (AT-294). The AT-90 T-DNA insertion site resides nearly 2 kb upstream of an encoded Zn(2)-Cys(6) protein that, based on Northern hybridization analysis, is expressed in wild-type apothecia but not in AT-90 apothecia. The encoded protein is not an ortholog of other Zn binuclear cluster proteins that have characterized roles in fungal development (i.e., NosA/Pro1; RosA). Apothecial development in this strain is initially arrested at stage 2 of disc morphogenesis (invaginated-stipe tip) and eventually bifurcates proliferatively or slowly expands to form a partially expanded fertile disc. The recovered insertion site of the AT-294 mutant resides 600 bp upstream of an encoded C2H2 type Zn finger DNA binding protein. The AT-294 mutant produces large numbers of fertile apothecia approximately one-tenth the size of wild type apothecia. The phenotype for the orthologous gene knockout in *Neurospora crassa* is reported in the *N. crassa* genome annotation as a lack of protoperithecia. Efforts to complement these *S. sclerotiorum* mutants will be reported.

450. *Aspergillus fumigatus* gene expression in experimental murine lung infections

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Aspergillus fumigatus is the most frequent cause of invasive aspergillosis in immune suppressed human patients. We have developed a murine model for analyzing the early stages of A. fumigatus colonization and progression to invasive disease. The model incorporates instillation of conidia into mouse lungs and subsequent harvesting of bronchoalveolar lavage fluid (BALF) samples for analysis. Validated mRNA amplification and analysis protocols have allowed transcriptome analysis of the fungal mRNAs present in the BALFs.

Expression profiling of *A. fumigatus* germlings at 12-14 hours after instillation into neutropenic mouse lungs reveals dramatically altered gene expression relative to growth in laboratory culture. Up-regulated genes are often found in secondary metabolism and other accessory gene clusters such as the gliotoxin, pseurotin, and siderophore biosynthesis clusters. We found also significant concordance between the observed host-adapted changes in the transcriptome and those resulting from *in vitro* iron limitation, nitrogen starvation, and loss of the LaeA methyltransferase.

To further elucidate the role of LaeA in *A. fumigatus* virulence, we analyzed temporal gene expression profiles of a wild type and an isogenic *laeA*-deleted strain, which misregulates gene expression at secondary metabolite gene clusters and is avirulent in a murine model. Growth and differentiation during initiating phases of murine infection were compared between parental and mutated isolates at 4, 8, and 14 hours post-infection in neutropenic mice. Transcriptome analysis of the *laeA* mutant revealed a major *in vivo* regulatory deficit of a few secondary metabolite biosynthetic gene clusters and more than thirty accessory gene clusters.

In our continuing studies employing this murine early infection model, we will undertake analysis of hypervirulent *A. fumigatus* mutants, *laeA* proteome analysis, and the murine host response to the fungal pathogen.

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451. The P-type ATPase, Apt1, is involved in a stress response and virulence in *Cryptococcus neoformans*

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Protein trafficking and secretion are important aspects of the pathogenicity of *Cryptococcus neoformans*. Secretion of virulence factors, such as capsule polysaccharide, is known to be mediated by exocytosis and specialized small vesicles. We have previously showed that capsule production can be blocked by trafficking inhibitors, and that some components of the protein trafficking pathway are regulated by cAMP-PKA signaling in this fungus. In the present study, we identified and characterized the gene *APT1* that encodes an integral membrane P-type ATPase belonging to the type IV, Drs2, family of aminophospholipid translocase (flippase) (APTs). APTs maintain the phospholipid asymmetry which is critical in membrane fusion events for protein trafficking. *APT1* in *C. neoformans* is functionally related to the Drs2 family of APTs. Deletion of *APT1* gene in a serotype A strain H99 results in the increased sensitivity to oxidative, salt and nitrosative stresses. The mutant (*apt1*) displays increased sensitivity to trafficking inhibitors such as Brefeldin A, a drug known to arrest the anterograde transport of proteins between the ER and Golgi apparatus, and monensin, a Na+/H+ ionophore that blocks intracellular transport in both trans-Golgi and post-Golgi compartments. The deletion mutant is also hypersensitive to the antifungal drugs amphotericin B and fluconazole. This result may be consistent with a role for Apt1 in lipid metabolism. There was no significant difference in growth, capsule formation or melanin production between the wild type and *apt1* mutant strains at either 30°C or 37°C. Deletion of the *APT1* homologue in CBS7779, a serotype A strain that showed disomy on chromosome 13, results in similar phenotypes. In a mouse inhalation model of cryptococcosis, *apt1* mutants exhibit significantly attenuated virulence compared to the wild type strain. We believe that *APT1* is likely involved in maintaining phospholipid asymmetry and ER/Golgi functions, thus contributing to the stress response and virulence in *C. neoformans*.

452. The LOV-Domain *SsVvd* protein from *Sclerotinia sclerotiorum* affects sclerotial development and virulence

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Light serves as an important signal in multiple developmental pathways of *Sclerotinia sclerotiorum*. We have identified a gene, *Ssvvd*, from *S. sclerotiorum* that encodes a member of the LOV domain protein family with greatest sequence identity with VVD/Envoy proteins. *Ssvvd* is expressed in all light-grown stages of *S. sclerotiorum*. In vegetative hyphae, transcript accumulation is observed within 15 min. and peaks around 30 min. of exposure to broad spectrum light. Two independent *Ssvvd* deletion mutants were created. When grown as radial colonies, the wild type produces large numbers of sclerotia at the colony periphery under all light conditions with fewer sclerotia produced in the dark. In contrast, the mutants produced large numbers of small sclerotia scattered over the colony surface under all light conditions and fewer sclerotia at the colony periphery in the dark. In race tube cultures grown under alternating 12 hr broad spectrum light and 12 hr dark, the wild type produces bands of aerial hyphae in the dark. Sclerotia are initiated by these aerial hyphae when exposed to light. In the *Ssvvd* mutants, bands of aerial hyphae are extended into the light- exposed phase of growth but fewer sclerotia are formed. Sclerotia of the *Ssvvd* mutants produce discontinuous rinds with mislocalized melanin deposition. These sclerotia fail to carpogenically germinate. Although no difference in colony growth rates are observed, *Ssvvd* mutants are reduced in virulence relative to wild type in tomato leaflet inoculation assays. Our findings suggest a role for SsVvd in photoregulation of sclerotia development in and indicate that additional roles during the life cycle of this broad host range necrotrophic plant pathogen.

453. *nepA* as a possible pathogenicity factor in *A. flavus*

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Infection of maize kernels by *Aspergillus flavus*, a pathogen of maize, results in kernel deterioration and contamination with aflatoxin. To identify putative pathogenicity genes, a custom-designed Affymetrix GeneChip DNA microarray was used to follow gene expression in *A. flavus* during infection of maize kernels in the field. Nearly 1,500 fungal genes were more highly expressed in infected living kernels when compared to expression in colonized autoclaved kernels at the same developmental stage. Of these, *nepA* was expressed on average 8 times higher in living kernels. *nepA* belongs to the necrosis inducing protein superfamily (NPP1), which several members in other plant pathogens are known to be involved in pathogenicity. To determine if *nepA* has a role in pathogenicity, the gene was deleted, and the mutant was used in pathogenicity tests. Growth and conidiation of the mutant on the kernel surface appeared sporadic and varied. In contrast, consistent differences in growth within kernels were observed between the mutant and wildtype . The *nepA* deletion mutant appeared to be impeded in growth in the endosperm, while wild type caused necrosis of kernel tissues. Additional experiments are being performed with beta-glucuronidase- expressing strains and histological stains to better define mycelium within kernel tissues. Our initial findings suggest that *nepA* has a role in the pathogenicity of *A. flavus*.

454. The role of *veA* on *Aspergillus flavus* infection of peanuts, corn and cotton.

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The aflatoxin-producing fungus *Aspergillus flavus* is a causal agent of preharvest contamination of food commodities such as oil seed crops worldwide. Peanut, corn and cottonseed are among the oil seeds that are susceptible to aflatoxin contamination during invasion of these crops by *A. flavus*. Contamination of agricultural commodities with aflatoxins poses serious negative economic and health impacts in both humans and domestic animals. It is therefore of a great importance to determine ways to control *A. flavus* dissemination, survivability and toxin formation. In this study we demonstrate the role of the *veA* regulatory gene in the infection of peanut, corn and cotton by *A. flavus*. Virulence of *A. flavus* on peanut and corn seeds was reduced in the absence of the *veA* gene product. Generation of air-borne asexual spores was reduced and production of aflatoxin and sclerotia in peanut seeds, viable or non viable, or in viable corn seed was completely blocked when infected with the *A. flavus veA* mutant (Delta-*veA*). In planta inoculation of cotton bolls also showed that conidiation was decreased in bolls inoculated with the Delta-*veA* strain and spread of the Delta-*veA* strain to seed in locules adjacent to the inoculated locule was less than observed with the wild-type *veA* strain. Our recent studies suggest that production of key enzymes necessary for infection of plant tissue is decreased in the Delta-*veA* compared to the control strain, particularly amylase production. As observed in peanuts and corn, no aflatoxin was produced in seed harvested from cotton bolls inoculated with the Delta-*veA* strain while aflatoxin was present in seed from wild-type *veA* inoculated bolls.

455. Functional analysis of Mps1 MAP kinase pathway in the rice blast fungus *Magnaporthe grisea*

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Cell wall integrity is crucial for fungal growth, development and stress survival. In yeast, Slt2 MAP kinase and calcineurin signaling pathways monitor cell wall repair during stress and development. MPS1 *M. grisea SLT2* orthologue, is also essential for appressorium mediated penetration into host plants (Xu 1998 PNAS 95:12713). Slt2 activates the transcription factors Rlm1, Swi4 and Swi6, while calcineurin activates Crz1 transcription factor. Genes orthologous to *CRZ1, MPS1, RLM1, SWI4, SWI6* were deleted in *M. grisea* by targeted gene replacement. Swi4 and Swi6 interact with Mps1 in yeast two hybrid assays. delta-mps1 mutants displayed abnormal mycelial growth, no sporulation, and lack of pathogenicity on plants as reported. delta-crz1, delta- rlm1, delta-swi6 mutants have growth and sporulation rates similar to wild type, and displayed a reduced pathogenicity on plants. delta-mps1 mutants are highly sensitive to nikkomycin Z (chitin synthase inhibitor), CFW (disorganization of cell wall) and aculeacine (glucan synthase inhibitor), while delta-crz1, delta-rlm1, delta-swi6 mutants are only partially hypersensitive to these fungicides. These studies suggest that transcription factors controlled by Mps1 are either functionally redundant or specialized in the control specific sets of cell wall repair target genes.

456. Novel fungal proteins in the chalkbrood infection of honey bee larvae.

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Discovery of novel fungal secreted proteins not only shed light on the biology of the secreting organism it may also lead to industrial exploitation. We expect that this is especially true for investigating the interaction between two organisms, which largely relies on secreted protein signals. Here we investigate the interaction between the honey bee and its fungal pathogen *Ascosphaera apis*, the causative agent of chalkbrood, by identifying enzymes secreted by bee and fungus during different timepoints of infection. Upon testing *A. apis*-infected larvae for enzyme activity, the larvae exhibiting significant activity were used to produce cDNA libraries. These dual organism cDNA libraries were then screened by transposon-assisted signal sequence trapping (TAST), a method well established for identifying genes for secreted proteins (Becker et al. (2004) J Microbiol Methods 57:123-133). After the trappants are sequenced and annotated, selected genes are further described. As a result, we will deepen the understanding of chalkbrood, one of the main honey bee pests with relevant impact on the economy, among others due to the essential role of bees in pollination.

457. Characterization of the apoptotic molecular mechanisms in *Cryptococcus neoformans*.

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Apoptotic-like cell death (ALCD) has been described in several fungi and is induced by different compounds and stress conditions. *C. neoformans* is an important opportunistic human pathogen and has been reported to undergo ALCD in response to co-culture with bacteria and to hydrogen peroxide. Conversely, capsular polysaccharides from *C. neoformans* are known to induce apoptosis in host immune cells, contributing to its virulence. It seems counterintuitive that an organism would both induce and undergo apoptosis in response to different signals, but it strongly indicates that the apoptotic machinery in *C. neoformans* has to be divergent enough from the host's in order to avoid its inappropriate activation in the context of pathogenicity. Hence, we propose to elucidate the apoptotic-signaling cascade in *C. neoformans* and to characterize its unique features when compared to the human machinery. We will confirm the observation that *C. neoformans* undergoes ALCD in response to oxidative stress and will further characterize other apoptotic inducing conditions in *C. neoformans*, such as stresses encountered in the environment and host. Finally, we will use genetic studies to define the genes and pathways required for *C. neoformans*' apoptotic response and investigate their suitability to be specifically targeted as a novel antifungal strategy.

458. The *Alternaria alternata* transcriptional factor AaAP1 involved in reactive oxygen species detoxification is a key virulence determinant on citrus.

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The production of reactive oxygen species (ROS), primarily superoxide and H_2O_2, plays an important role in many plant-microbial interactions. The tangerine pathotype of *Alternaria alternata* is a necrotrophic fungal pathogen of citrus, whose pathogenicity mainly depends on host-selective ACT toxin. We characterized a homolog of the yeast YAP1 transcription factor, designed AaAP1, from *A. alternata* and demonstrated an important role for detoxifying ROS in fungal pathogenicity. The *A. alternata* AaAP1 contains all conserved domains required for cellular localization of YAP1 and for YAP1-mediated resistance to oxidative damages. Deletion of the *AaAp1* gene in *A. alternata* resulted in an increased sensitivity to H_2O_2, menadione, and tert-butyl hydroperoxide, and a loss of fungal pathogenicity. The *AaAp1*-disrupted mutants also displayed marked reduction in catalase, peroxidase, and SOD activities, whereas deletion of the *AaAp1* gene did not affect conidial formation or production of ACT- toxin. The *AaAp1* null mutants failed to establish colonization in Minneola leaves, even within the pre-wounded tissues. All mutant phenotypes were restored in fungal strains acquiring and expressing a wild-type copy of *AaAP1*. Application of NADPH oxidase inhibitors partially restored lesion formation by the *AaAP1*-disrupted mutants. Taken together, we conclude that *AaAp1* is essential for *A. alternata* pathogenesis. Our study also highlights the absolute requirement of oxidative stress response in *A. alternata* for successful colonization to its host plant.

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459. Nitrogen controls invasive growth and plant pathogenicity in *Fusarium oxysporum* via the Ser/Thr kinase TOR and the bZIP transcription factor MeaB.

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During infection, fungal pathogens activate virulence mechanisms such as host adhesion, penetration and invasive growth. In the vascular wilt fungus *Fusarium oxysporum*, a mitogen-activated protein kinase (MAPK), Fmk1, was shown to be required for efficient root adhesion, penetration of cellophane sheets and plant infection. Here we studied the role of nitrogen regulation in the control of these virulence functions. Root adhesion and cellophane penetration were strongly impaired in presence of the preferential nitrogen source ammonium. By contrast, *F. oxysporum* mutants lacking MeaB, a bZIP transcription factor that mediates nitrogen metabolite repression in *Aspergillus*, still performed root adhesion and cellophane penetration in the presence of ammonium. Deletion of *meaB* did not restore root adhesion and cellophane penetration in a *fmk1* mutant, suggesting that MeaB and Fmk1 regulate these virulence functions through separate pathways. Interestingly, tomato plants supplied with ammonium, rather than nitrate, showed a significant reduction of vascular wilt symptoms when infected by the wild type strain, but not the *meaB* mutant. Rapamycin, a specific inhibitor of the conserved Ser/Thr kinase TOR, restored adhesion and cellophane penetration of *F. oxysporum* in the presence of ammonium. Two other plant pathogens, the rice blast fungus *Magnaporthe grisea* and the wheat head blight fungus *F. graminearum*, also showed repression of cellophane penetration by ammonium. Our results suggest that a conserved nitrogen response pathway operating via TOR and MeaB controls a subset of virulence functions in plant pathogenic fungi.

460. MGOS: Development of a Community Annotation Database for *Magnaporthe oryzae*.

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Magnaporthe oryzae the rice blast pathogen is a significant and persistent problem to rice cultivation. The MGOS database (www.mgosdb.org) was developed to provide data on the interaction between host (rice) and pathogen including genome sequence, expression data and mutant information. It provides a systematic approach to explore the results along with genome browsers for M. oryzae genomic sequences. To extend the utility of MGOS, we have created a manual annotation feature to allow the researchers to enter all of their mutant, sequence and expression information. MGOS contains the Broad Institute's version 6 and 5 gene models as well as its own gene models. While developing the database and a manual describing how to use it, we have manually annotated over 496 genes, associating gene names, transcripts and literature citations (149) with them as well as any phenotypic data based on mutant studies. Each gene also has interpro identification associated with it, and one can BLAST genes directly against MGOS or NCBI. For manual curation of genes, options include adding gene symbols, synonyms, gene names, transcripts, GO terms, fungal anatomy terms, publications, and mutant information. MGOS also allows addition of gene information from strains other than 70-15, so that the data relates to the species, not just the strain whose genome was sequenced. We encourage the community to input new data in MGOS.

461. Exploring *P.infestans* effector functions using the yeast eukaryotic model.

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Phytophthora species include some of the most devastating plant pathogens known. During infection, these oomycete pathogens secrete an armada of effector proteins that neutralize host defense systems, including the hypersensitive response, a form of programmed cell death. Many of the secreted *Phytophthora* effectors seem to be directed into the host cell by the N-terminal amino acid motif RXLR in combination with downstream acidic residues. Bioinformatics analysis has revealed that *Phytophthora* genomes may encode hundreds of these RXLR effectors. Outside of the N-terminal signal domain, however, RXLR effectors rarely exhibit homology to each other or to previously characterized proteins; consequently, their roles in infection remain largely unknown. Recently researchers have demonstrated that the function of bacterial effectors that target conserved eukaryotic pathways can be elucidated using yeast functional genome screens. Therefore, to discern the function of putative host cell- targeted effectors, we have begun by expressing a panel of *P. infestans* RXLR effectors using low- and high-copy number yeast expression vectors. Currently we are assaying each RXLR effector for its ability to suppress programmed cell death or to inhibit growth in yeast. Subsequently, yeast functional genomic screens can be used to identify the conserved targets of effectors that compromise yeast growth.

462. Understanding sex in a hostile environment - cryptic mating in *Candida albicans*

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The opportunistic human pathogen Candida albicans was, until recently, thought to be an obligate asexual fungus. A cryptic mating cycle has been uncovered, however, in which efficient mating requires that cells first undergo a phenotypic switch from the 'white' state to the mating competent 'opaque' state. This novel form of mating regulation is thought to limit C. albicans mating to specific host niches. Evidence from the laboratory demonstrates that the white-opaque switch is highly sensitive to diverse environmental stimuli, including oxidative and genotoxic stress, as well as genetic manipulation of strains. We show that these apparently diverse factors influence the rate of phenotypic switching via an effect on the rate of cell growth. A model is discussed whereby changes in growth rates alter the frequency of switching by modulating Wor1 protein levels – the central protagonist for formation of the opaque state in C. albicans. Increased phenotypic switching in response to stressful environments may be beneficial to the organism as it can directly promote escape from host defenses, as well as directing entry into the program of sexual reproduction.

463. A novel pathogenicity gene is required in the rice blast fungus to suppress the basal defenses of the host.

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For successful colonization and further reproduction in host plants, pathogens need to overcome the innate defenses of the plant. We demonstrate that a novel pathogenicity gene, *DES1*, in *Magnaporthe oryzae* regulates counter-defenses against host basal resistance. The *DES1* gene was identified by screening for pathogenicity-defective mutants in a T-DNA insertional mutant library. Bioinformatic analysis revealed that this gene encodes a serine-rich protein that has unknown biochemical properties, and its homologs are strictly conserved in filamentous Ascomycetes. Targeted gene deletion of *DES1* had no apparent effect on developmental morphogenesis, including vegetative growth, conidial germination, appressorium formation, and appressorium-mediated penetration. Conidial size of the mutant became smaller than that of the wild type, but the mutant displayed no defects on cell wall integrity. The delta-*des1* mutant was hypersensitive to exogenous oxidative stress and the activity and transcription level of extracellular enzymes including peroxidases and laccase were severely decreased in the mutant. In addition, ferrous ion leakage was observed in the delta-des1 mutant. In the interaction with a susceptible rice cultivar, rice cells inoculated with the delta-*des1* mutant exhibited strong defense responses accompanied by brown granules in primary infected cells, the accumulation of reactive oxygen species (ROS), the generation of autofluorescent materials, and PR gene induction in neighboring tissues. The delta-*des1* mutant displayed a significant reduction in infectious hyphal extension, which caused a decrease in pathogenicity. Notably, the suppression of ROS generation by treatment with diphenyleneiodonium (DPI), an inhibitor of NADPH oxidases, resulted in a significant reduction in the defense responses in plant tissues challenged with the delta-*des1* mutant. Furthermore, the delta-*des1* mutant recovered its normal infectious growth in DPI-treated plant tissues. These results suggest that DES1 functions as a novel pathogenicity gene that regulates the activity of fungal proteins, compromising ROS-mediated plant defense.

464. A dual 'omics approach to characterise asexual sporulation and toxin production in *Stagonospora nodorum*

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Stagonospora nodorum is a major fungal pathogen of wheat. It has been previously shown by this laboratory that mutants defective in heterotrimeric G protein and MAPK signalling were affected in pathogenicity and showed developmental defects. In this study, we sought to gain a better understanding of signal transduction and phytopathogenicity in *S. nodorum* through the identification and characterisation of signalling target proteins. A comparative analysis of the *S. nodorum* wildtype and signalling mutants with 2-DE proteomics has led to the identification of a putative short- chain dehydrogenase (Sch1) that is subjected to positive regulation by both signalling pathways. Real-time PCR and transcriptional GFP fusion expression analyses have revealed that the *Sch1* is strongly expressed during asexual sporulation. Mutants lacking *Sch1* were altered in vegetative growth and showed a strong reduction in asexual sporulation. Detailed histological studies of the *sch1* mutants revealed a role for gene in facilitating the development of the fertile sub-parietal layer of asexual pycnidia and consequent sporulation. In addition, comprehensive non-targeted metabolomic analyses of the sch1 mutants identified the strong accumulation of a metabolite positively identified as the mycotoxin alternariol. This is a first report that confirms the presence of a post-harvest mycotoxin in *S. nodorum*.

465. Development of a screen for the identification of *Aspergillus* adhesins.

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The first stage of invasive Aspergillosis is the binding of air borne conidia to pulmonary epithelial cells and extracellular matrix proteins. Conidia of *Aspergillus nidulans* bind to buccal epithelial cells. *A. nidulans* conidia do not bind to glass but do bind to fibronectin, a key component of extracellular matrix, when it is used to coat surfaces of glass and magnetic beads. Treatment of *A. nidulans* conidia with trypsin abrogates binding to fibronectin. Binding of *S. cerevisiae* to fibronectin is dependent upon expression of the cell surface flocculin Flo11p. S288C, a Flo11-deficient strain, does not bind to fibronectin coated surfaces. However, expression of Flo11p in S288C restores binding to fibronectin. An *Aspergillus* cDNA library could thus be screened for fibronectin-binding adhesins in S288c *S. cerevisiae*. To assess the validity of using this method to identify genes encoding adhesins, four genes encoding potential adhesion proteins, two from *A. nidulans*, *mnpA* and *ypsA*; and two from *A. fumigatu*s, *AFMP1* and *AFMP2* were expressed in S. cerevisiae S288C. Transformants were tested for binding to fibronectin coated wells in microtiter plates. Of the four, only expression of *AFMP*2, a putative *A. fumigatus* cell wall mannoprotein (1) partially restored binding of S288C to fibronectin-coated plastic. (Supported in part by NIGMS R15GM077345-01A1.)

466. Functional analysis of genes encoding proteins secreted by *Pyrenochaeta lycopersici* during infection of tomato roots

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Our goal is to study the role of proteins secreted by the filamentous ascomycete *Pyrenochaeta lycopersici* during the infection of tomato roots. We are combining a genetic approach using the yeast trap system (1) with the purification of extracellular proteins having biological activity that could be related with infection. We have recently identified a putative exoglucanase, Pl-EXO, homologous to EXG1 of *Cochliobolus carbonum* in axenic culture filtrates of the fungus and we are currently purifying to homogeneity a protein with an HR-inducing activity which is specific of tomato. In order to characterize later those who could be required for the development of corky root rot disease, we are testing reverse genetics strategies with two genes of *P. lycopersici* already cloned in our laboratory: *Pl-EXO* and *Pl-FOW1* (encoding a putative mitochondrial carrier protein homologous to *FOW1* from *Fusarium oxysporum*). Gene replacement of *Pl-FOW1* has been tested using large genomic flanking regions together with the selectable marker for resistance to hygromycin, and we are currently testing RNA interference of both genes using the pSilent-1 vector (2). (1)Lee et al. 2006 Mol. Plant Microbe-Interact. 19(12):1368 (2)Nakayashiki et al. 2005 Fungal Genet. Biol. 42:275

467. Ammonia secretion act as an elicitor signal and as a pathogenicity factor

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Secretion of ammonia is known to be associated with virulence of the pathogenic fungi *Colletotrichum coccodes*. Consistent with this, we have developed nit- and areA mutants that are compromised in ammonia secretion and are less virulent. While at tomato fruit colonization, ammonia causes alkalinization and a subsequent increase in fungal pathogenesis factors, we examined if ammonia can also directly contribute to fungus pathogenicity while keeping an acidic pH. Treatment of the infection court with low pH buffer was found to elevate total ammonia secretion and increase aggressiveness in comparison to with high pH treatment. This indicates that the effect of ammonia secretion goes beyond direct pH control. Indeed, we demonstrate that ammonia activates the tomato host membranal NADPH oxidase (Rboh) and stimulates ROS accumulation. Direct application of ammonia to plant tissue without infection leads to membrane leakage and local host cell death. The results suggest that activation of the membranal Rboh by ammonia is important for fungal virulence of this necrotroph pathogen. Indeed, tomato Rboh mutants produce less ROS and showed less ammonia-induced-ion- leakage and less cell death. As necrotrophic fungus prospers by host cell death, the subversion of Rboh activity by the fungus through ammonia secretion is a novel virulence strategy. This work demonstrates that *Colletotrichum coccodes* uses ammonia secretion as a novel dual-edged strategy; optimizing the infection court pH which is conducive to fungal virulence gene activation and at the same time stimulating host ROS production and local cell death.

468. *Fusarium graminearum* proteins important in host interaction.

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We used proteomics to analyze the secretome of *Fusarium graminearum* (Fg) *in vitro* and *in planta*. Secreted proteins from Fg grown *in vitro* on different carbon sources or extracted from infected wheat heads by vacuum infiltration were digested en masse with trypsin and analyzed by LC-MS/MS. From *in vitro* cultures, ~230 proteins could be identified with high statistical probability. Despite the preponderance of host proteins in infected wheat heads, 120 fungal proteins were identified, many predicted to interact with host cell walls. Although >85% of the *in vitro* proteins were predicted to have signal peptides, only 61% of the *in planta* proteins were. Among the proteins lacking signal peptides found *in planta* were 13 housekeeping enzymes. The presence of these proteins in the *in planta* secretome might be due to fungal lysis, but several of them have been reported to be immunogens secreted by animal pathogenic fungi. These proteins might therefore have a role in the interaction between *F. graminearum* and its host. We are currently testing these housekeeping proteins separately, as well as the proteins collected in culture filtrates from Fg grown on corn cell walls to determine if any act as virulence factors or pathogen associtated molecular patterns (PAMPs) to trigger the innate immune response in non-host plants. Low concentrations of Fg proteins applied to *Arabidopsis* grown in liquid culture cause stunted seedling growth. Interestingly, this response is inhibited when the filtrate is boiled, suggesting a protein component. This active component is currently being isolated.

469. High resolution analysis of fungal secreted proteins.

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Fungal-plant interactions are widespread. For instance, most tree species develop symbiotic relationships with fungi to enhance nutrient absorption, whilst many crop diseases that result in massive yield losses are caused by fungi. Little is known about proteins involved in these associations, but secreted proteins (secretomes) are predicted to play a key role. We present the first secretome analysis of fungi interacting with plants, the tree symbiont *Laccaria bicolor* and the rapeseed pathogen *Leptosphaeria maculans*. In this analysis, gel-based and gel-free proteomic approaches combined to mass spectrometry (MS) are exploited. Fungi grown in liquid media, with or without plant extracts were analysed. Fungal secreted proteins were recovered from medium filtrates. Several extraction methods were tested in combination with three proteomic techniques: two-dimensional electrophoresis (2-DE) followed by MS analyses, isoelectric focusing (IEF) followed by MS analyses (IPG shotgun) and one-dimensional electrophoresis followed by MS analyses (SDS-PAGE shotgun). The best 2-D gel resolution was achieved through an initial pre-fractionation using liquid-phase IEF followed by classic IPG-IEF/SDS-PAGE with narrow pH ranges. Hundreds of 2-D spots were thus resolved. In both species most of the secreted proteins had unknown functions. Of the ones with matches, most of them were to enzymes involved in cell wall modification or protein metabolism. This study paves the way for *in vivo* experiments and characterization of the fungal extracellular proteins involved in plant- fungus interaction.

470. Ectomycorrhizal gene expression during symbiosis and in heavy metal response

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We have identified genes specifically expressed in host interaction using an RNA fingerprinting technique. These genes of compatible *versus* low compatibility interactions of *Tricholoma vaccinum* with *Picea abies* or *Pinus sylvestris* can can subsequently be used to identify host signals. From 145 bands separated on agarose gels, 52 % were differentially expressed. 130 fragments were cloned, 57 of which were mykorrhiza-specific. Of 23 fungal genes with mycorrhiza-specific expression, sequence analyses were performed in order to identify the nature of the encoded protein *in silico*. Examples for potentially relevant, ecological function include aldehyde and alcohol dehydrogenases, an APS kinase, two MATE transporters and Ras. Additionally, two different classes of retrotransposon were identified which is the first identificaiton of actively expressed trnasposons in ectomycorrhizal fungi which might be the reason for high morphological diversity observed with *T. vaccinum*. The response of T. vaccinum to heavy metal stress was analyzed and genes up- regulated during heavy metal exposition were screend. The role in ectomycorrhizal symbiosis is investigated in a natural oak forest, where different morphotypes and exploration types of ectomycorrhizal fungi can be linked to different successional stages and different metal contamination.

471. High temperature and iron limitation: functional analysis of *Cryptococcus neoformans* and *Cryptococcus gattii* genes related to host-pathogen interactions.

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The expression of pathogens virulence determinants are highly controlled by the host milieu. Thus the description of crucial genes for host-pathogen interaction is fundamental for a better understanding of virulence mechanisms utilized during infection. The yeasts *C. neoformans* and *C. gattii* are closely related species that cause meningoencephalitis. We applied RDA to search for *C. neoformans* and *C. gattii* upregulated genes under two conditions that mimic host-pathogen interaction: growth at 37 ^{0}C or iron limitation. In the *C. neoformans* temperature library, we found 29 genes with higher expression involved in distinct biological processes, such as filamentation (glyoxal oxidase precursor) and transport (calcium ion transporter). In the *C. gattii* iron limitation libraries, thirty six genes were found to be upregulated. One of them is ortholog of a *Candida albicans* transcriptional regulator involved in nitrogen metabolism. These three genes were chosen for functional analysis. The biolistic transformations are being conducted in *C. neoformans* (H99) and *C. gattii* (R265) to generate null mutants. The virulence of these strains will be evaluated in an experimental model of cryptococcosis. These findings will contribute to define essential genes for virulence mechanisms utilized during *C. neoformans* and *C. gattii* host infection.

472. Conidiation color mutants of *Aspergillus fumigatus* are highly pathogenic to the heterologous insect host *Galleria mellonella*

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Invertebrates have been increasingly viewed as a valid model for virulence studies of human fungal pathogens as their virulence traits are likely conserved among different hosts. The caterpillar *Galleria mellonella* has been used as a heterologous host for a number of yeast pathogens. Here we have evaluated the possibility of applying this heterologous insect model to investigate the virulence trait of the filamentous fungal pathogen *Aspergillus fumigatus*: melanization. Melanization in *A. fumigatus* confers bluish-grey color to conidia and is a known virulence factor in mammal models. Surprisingly, conidial color mutants with deletions in the defined melanin biosynthesis gene cluster in B5233 background caused enhanced insect mortality. Insertional mutants in Af293 background producing conidia of previously identified colors and of novel colors were isolated and they also displayed a higher level of pathogenicity in the insect model, confirming the relationship between fungal melanization defects and enhanced virulence to the caterpillar. Exacerbated insect immune response induced by increased exposure of PAMPs and elevated levels of fungal secreted metalloproteinases may cause the increased mortality of the larvae infected with the color mutants. Our study underscores the importance of the knowledge about the insect innate immunity status in understanding fungal pathogenicity in insect models. This study also shows that the G. mellonella is a reproducible model for *A. fumigatus* that could become a valuable tool for studying fungal traits that are required for infections in both mammals and the insect. Additionally, our observations indicate the potential of using melanization defective mutants of natural insect fungal pathogens in the biological control of insect populations.

473. A sterol-regulatory element binding protein is required for cell polarity, hypoxia adaptation, azole drug resistance, and virulence in *Aspergillus fumigatus*.

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Aspergillus fumigatus is a saprophytic fungus commonly found in soil and compost piles. In immunocompromised patients it takes on a sinister form as a potentially lethal opportunistic human pathogen. At the site of infection, the significant influx of immune effector cells and the necrosis of tissue by the invading pathogen generates a hypoxic microenvironment in which both the pathogen and host cells must survive. Currently, whether hypoxia adaptation is an important virulence attribute is unknown. Here we report the characterization of a sterol-regulatory element binding protein, SrbA, in *A. fumigatus*. Loss of SrbA results in a mutant strain of the fungus that is incapable of growth in a hypoxic environment and consequently incapable of causing disease in two distinct murine models of invasive pulmonary aspergillosis (IPA). Transcriptional profiling and annotation of genes that are affected by loss of SrbA function implicated that SrbA is involved in maintaining sterol biosynthesis and cell polarity. Further examination of the SrbA null mutant phenotype revealed that SrbA plays a critical role in ergosterol biosynthesis, resistance to the azole class of antifungal drugs, and in maintenance of cell polarity in *A. fumigatus*. Significantly, the SrbA null mutant was highly susceptible to fluconazole and voriconazole. These findings present a new function of SREBP proteins in filamentous fungi, and demonstrate for the first time that hypoxia adaptation is an important virulence attribute of pathogenic molds.

474. Physical-chemical plant-derived signals induce differentiation in *Ustilago maydis*

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Ustilago maydis is able to initiate pathogenic development after fusion of two haploid cells with different mating type. Cell-cell recognition is based on a pheromone/receptor system. The pheromone signal is transmitted via a MAP kinase module leading to the activation of the Prf1 transcription factor, the essential regulator of sexual and pathogenic development. On the maize leaf surface, the resulting dikaryon switches to filamentous growth, differentiates appressoria and penetrates the host. Here we report on the plant signals required for filament formation and appressorium development in *U. maydis*. With the help of a marker gene that is specifically expressed in the tip cell of hyphae forming appressoria, in vitro conditions were established for filamentation as well as appressorium differentiation. Using a solopathogenic strain that is able differentiate without a mating partner, we show that hydroxy-fatty acids stimulate filament formation. These filaments resemble conjugation tubes. We show that hydroxy-fatty acids stimulate the induction of pheromone genes and this signal then activates the MAP kinase module.The hxdroxy-fatty acid signal can be bypassed by genetically activating the downstream MAP kinase module. Hydrophobicity also induces filaments and these resemble the dikaryotic filaments formed on the plant surface. When both signals are combined, about 30 % of the filaments develop appressoria. These results show that the early phase of communication between *U. maydis* and its host plant maize involves two distinct stimuli.To obtain insight into perception and downstream signaling after exppsure to hydrophobicity and/or hydroxy- fatty acids we performed microarray experiments using the artificial system. These results will also be presented.

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475. The Trehalose Pathway is critical for *Aspergillus fumigatus* virulence

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Trehalose, a disaccharide sugar, accumulates in *Aspergillus* conidia and plays a role in protection against stress including: high temperature, high osmolarity and ROS. In this study, we have characterized the trehalose pathway in *A. fumigatus* for the first time. Phylogenetic analyses revealed multiple copies of the trehalose biosynthesis gene *tps1* (*tpsA* and *tpsB*), a single copy of the trehalose-6-phosphate (T6P) phosphatase *tps2* (*orlA*), and single copies of *tps3* and *tsl1* orthologs. We have generated single and double null mutants of *tpsA* and *tpsB* and show that both genes are required for trehalose biosynthesis. Generation of a single *tpsA* or *tpsB* mutant did not alter trehalose accumulation, while generation of a *tpsA tbsB* double mutant completely abolished trehalose biosynthesis. Generation of an *orlA* (*tps2*) null mutant revealed a role for *orlA* in asexual conidiation when cultured on glucose minimal media. However, the defect in conidiation could be recovered on both sorbitol and glycerol minimal media. The *orlA* and *tpsA tpsB* mutants displayed sensitivity to growth at high temperatures (50ºC). Surprisingly, lack of *orlA* did not affect the production of trehalose at 37ºC suggesting an alternate pathway for trehalose biosynthesis exists in *A. fumigatus*. Of particular interest, the *orlA* null mutant was avirulent in two murine models of invasive aspergillosis. Our results suggest that trehalose and T6P are important components of the biology of *A. fumigatus*. Importantly, our results suggest that T6P phosphatase contributes to the ability of this mold to cause lethal disease in immunocompromised patients. Thus, as others have suggested, the trehalose pathway is worth exploiting as an antifungal drug target given its conservation in other pathogenic fungi and absence in humans.

476. Virulence determinants in *Cryptococcus gattii*: identification of micronutrient homeostasis and adhesion related genes through insertional mutagenesis.

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The microorganism development in the host milieu is highly dependent of nutrients availability. Microelements (e.g. iron, copper and zinc) are fundamental for the microorganisms, mainly as cofactors of enzymes involved in several biological processes. In the yeast *Cryptococcus neoformans*, iron metabolism is the target of several studies and show to be fundamental to development and virulence. However, in *C. gattii*, no reports of genes involved in micronutrients metabolism were described. In order to elucidate the molecular mechanisms that controls the micronutrients homeostasis in *C. gattii* R265 strain, we generated a T-DNA mutant library. Three distinct iron related phenotypes of this mutant library are being screened: (i) TTC reduction; (ii) BPDS-Ferrous precipitation; (iii) growth defects in zinc overload. Up to now, from 8,800 mutants, three were selected due to its high reductant activity over TTC. Apart from the search for genes involved in micronutrient metabolism, we are looking for genes that control the adhesion to pulmonary epithelial cells. Interactions assays between the mutant library and the A549 adenocarcinoma strain were being conducted to evaluate genes involved in adhesion. The genes inactivated are being identified and will be functionally characterized.

477. Appressorium development in the smut fungus *Ustilago maydis* is regulated by Sho1 and Msb2 related proteins.

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The plant pathogen *Ustilago maydis* causes smut disease on maize. On the plant surface, this dimorphic fungus switches from yeast-like budding to filamentous growth and develops a specialized infection structure called appressorium. Recent work identified hydrophobic surface and cutin monomers as environmental cues which induce appressorium formation in *U. maydis*. Two hybrid screening revealed a homolog of Sho1 interacting with the MAP kinase Kpp6, an essential component for plant cuticle penetration. In *Saccharomyces cerevisiae* Sho1p acts together with Msb2p to regulate osmotic stress response and pseudohyphal growth. In *U. maydis* we identified a transmembrane mucin with similarity to Msb2p. In single *sho1* or *msb2* mutants virulence was reduced and microscopic analysis revealed decreased amounts of appressoria on the leaf surface of infected plants. *sho1*/*msb2* double deletion mutants had lost their ability to develop appressoria and consequently the infected plants did not develop disease symptoms. Targeted expression of a constitutive active allele of the MAPKK Fuz7 restored appressorium development in this mutant. In vitro studies showed that *sho1* and *msb2* mutants responded to cutin monomers but were impaired in their filamentation response induced by the hydrophobic stimulus. *sho1* and *msb2* were not required for osmosensing and other stress responses. Our data suggest that Sho1 and Msb2 are plasma membrane receptors that regulate appressorium development in response to hydrophobic surfaces by activating a MAP kinase cascade.

478. The *pit* gene cluster: an effector and a transmembrane protein are crucial determinants for virulence.

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Biotrophic plant pathogenic fungi develop an intimate relationship with their hosts, rather than killing the host like necrotrophic pathogens. *Ustilago maydis* - a smut fungus that infects maize plants - serves as a model system to study such a sophisticated host- pathogen interaction. Previous genomic analysis revealed that *U. maydis* codes for a large set of novel, small, secreted protein effectors. A significant number of the respective genes are arranged in clusters that are induced during plant infection. The deletion of several of these clusters dramatically affected virulence (Kaemper *et al.* 2006, Nature 444:97-101). Here we present ongoing work on a newly identified gene cluster encoding a small, secreted protein (Pit2) and a 57 kDa transmembrane protein (Pit1). The expression of *pit1* and *pit2* was restricted to the biotrophic growth phase. Deletion of the entire cluster as well as single deletions of either *pit1* or *pit2* leads to the loss of virulence. Both mutants were able to infect maize plants and could proliferate in meristematic tissue but failed to induce tumors. Using confocal microscopy we have been able to localize GFP-tagged versions of both proteins during the biotrophic phase. The effector Pit2 appeared to be secreted from the fungal hyphae whereas the transmembrane protein Pit1 localized to three distinct compartments: the GFP-signal could be detected in the plasma membrane at the hyphal tips, within the vacuolar lumen and, interestingly, also on moving particles. Using an endosomal marker protein we detected partial co-localization of Pit1-GFP and early endosomes. We presume that there exists a functional link between Pit1 and Pit2. In order to establish such a link we are analyzing whether Pit1 and Pit2 interact, identify putative additional interaction partners and perform physiological studies to address the putative functions of both proteins.

479. Evaluation of gibberellin production by the basidiomycete *Moniliophthora perniciosa*, the causal agent of Witches' Broom Disease in cacao.
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Moniliophthora perniciosa is a hemibiotrophic fungus and the causal agent of Witches' Broom Disease (WBD) of cacao. The typical symptoms of WBD (hyperplasia, hypertrophy and loss of apical dominance in infected tissues) historically have been correlated to a hormonal imbalance. The *M. perniciosa* Genome Project has led to the identification of putative genes homologues to all genes involved in the gibberellin (GA) biosynthesis pathway of *Gibberella fujikuroi*. Based on these findings, we hypothesized that *M. perniciosa* could produce GA. Thus, the present work aimed to verify this hypothesis through biochemical and genetic analyses. This is the first report of the existence of a putative Copalil diphosphate synthase/ent-kaurene synthase (CPS- KS) gene that codes for the first specific enzyme of the GA biosynthesis pathway in a basidiomycete. The CPS-KS gene was cloned and expressed in bacteria in order to verify the biological activity of the protein. Also, the CPS-KS gene expression profile was analyzed throughout the life cycle of the fungus by real-time RT-PCR suggesting that the enzyme is more expressed in the infective phase of this fungus. Moreover, using a combination of Thin-Layer Chromatography and Mass Spectrometry techniques fungal spores extract was analyzed and a substance with the same molecular weight of GA3 has been already identified. We conclude that *M. perniciosa* is likely to produce GA as a pathogenic factor, and that this hormone may play a key role in the development of WBD.

480. *Fusarium* spp. associated with field-grown grain of near-isogenic low lignin and wild-type sorghum.
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Previous studies indicated that low lignin brown midrib (bmr) sorghum may be more resistant to *Fusarium* spp. than wild-type and that phenolic profiles of near- isogenic plants varied depending on bmr genotype. *bmr*-6 and *bmr*-12 were backcrossed into wild-type sorghum backgrounds, resulting in reduced lignin near- isogenic genotypes. Colonization by *Fusarium moniliforme* sensu lato of *bmr*-6 and *bmr*-12 field-grown grain was significantly reduced when compared with that of wild-type grain. Sequence analysis of the translation elongation factor gene showed that *F. thapsinum* was the most frequently isolated *Fusarium* species with *F. bullatum, F. proliferatum* and *F. pallidoroseum* also commonly recovered. Sixty-five percent of isolates from *bmr*-12 grain were *F. thapsinum*, while 44% and 40% of isolates from *bmr*-6 and wild-type, respectively, were this species. *F. bullatum* isolates were readily recovered from wild-type and *bmr*-6 grain, but no such isolates were detected in *bmr*-12 grain. Lesions produced on *bmr*-12 plants following inoculation with *F. thapsinum* were significantly smaller than those on wild-type plants, while inoculation with *F. armeniacum* or *F. verticillioides* produced smaller lesions on *bmr*-6 plants as compared with wild-type. These data suggest that differences in accumulation of phenolic precursors in bmr plants may affect colonization by *Fusarium* genotypes.

481. Towards a fully description of *Fusarium graminearum* infection process at the molecular level
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The filamentous fungus *Fusarium graminearum* is the most common pathogen of the *Fusarium* head blight for wheat and the *Gibberella* stalk rot for maize. It is one of the most destructive plant pathogens for the world agriculture. We still do not clearly understand how the *Fusarium graminearum* interacts with plant and how *Fusarium graminearum* causes the disease at molecular and cellular levels. Given that the genome sequence of *Fusarium graminearum* has been available recently, genome-wide in planta expression profiling of *Fusarium graminearum* can help us fully describing the disease infection. We are exploring the possibility of using laser capture microdissection to isolate different infection stages of *Fusarium graminearum* in wheat and maize for fungal microarray analysis. Furthermore, we are using high through-put RNA interference strategy to screen for pathogenicity genes important for *Fusarium graminearum* infection of plants.

482. Involvement of the transcription factor *snt2* in pathogenesis and development of *Fusarium oxysporum* f. sp. *melonis*.
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The soil-borne pathogen *Fusarium oxysporum* f. sp. *melonis* (FOM) causes vascular wilt disease of muskmelon. Using an *Agrobacterium*-mediated tagged-mutagenesis approach, we isolated a FOM mutant (D122) which exhibits a reduction of 80% in plant mortality, when compared to the wild type. The defect in D122 was attributed to an insertion in the gene encoding a *snt2*-like transcription factor (TF), which harbors two PHD fingers and a BAH domain. Subtractive Suppression Hybridization was used to compare expression profiles between the wild type and D122 isolates. One of the differentially-expressed genes encodes a putative b-ZIP TF whose *Podospora anserina* homolog (*idi-4*) is involved in execution of programmed cell death. Quantative RT-PCR showed that *idi-4* expression was 32-fold higher in D122 and in a delta *snt2* strain, when compared to the wild type, suggesting that *snt2* may be a novel negative regulator of *idi-4*. Both D122 and delta *snt2* showed reduced radial growth and a significant reduction in conidial production, demonstrating that *snt2* is also involved in fungal development. In addition, deregulation of SOD expression was observed in the *snt2* strain. Elucidating the links between *snt2* function and other regulatory pathways may provide a better understanding of fungal pathogenicity and development.

483. Delving into the mechanism of LaeA regulated secondary metabolism
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LaeA, a global regulator of secondary metabolism within the aspergilli and related fungi, has been shown to be important for pathogenicity in a model of invasive aspergillosis. Null mutants of LaeA are nearly avirulent in the murine model and also show increased phagocytosis by alveolar macrophages *in vitro*. LaeA has recently been shown to be part of a protein complex, termed the velvet complex, which coordinates sexual development and secondary metabolism in the model organism *A. nidulans*. While LaeA control of secondary metabolism appears to be conserved within the genus *Aspergillus*, its function remains an enigma. We are interested in determining what makes LaeA contribute to pathogenicity in *A. fumigatus*. Towards this end, we have constructed a normalized yeast-two hybrid cDNA library to screen for protein interactors of LaeA. Additionally, we have utilized an AMA1 genomic library of *A. fumigatus* (AF293) to look for multi-copy suppressors of *?laeA*. By employing two forward genetic screens in the pathogenic mold *A. fumigatus*, we have putatively identified several loci that may be involved in LaeA regulated pathogenicity factors.

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484. The immune response of brown algae against the basal oomycete pathogen *Eurychasma dicksonii*

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The oomycete pathogen *Eurychasma dicksonii* is both the most abundant eukaryotic pathogen of marine brown algae, and the most basal member of the oomycete lineage. Despite being an obligate biotroph, it has the largest reported host range among marine pathogens - infecting virtually every brown algal species tested so far. Remarkably, virtually nothing is known about many fundamental aspects of its pathogenicity, biology, epidemiology, and ecology. Due to its availability in culture and the recently-completed sequencing of the genome of one of its main brown algal hosts (*Ectocarpus siliculosus*), *Eurychasma* is a particularly attractive model to study oomycete infection strategies and algal defense mechanisms. The reaction of different algal strains against *Eurychasma* range from extreme susceptibility to complete resistance against infection, suggesting a genetic basis for disease resistance in algae. In all cases investigated, resistance is associated with the early death of the challenged algal cell, which prevents further spread of the disease. This holds true across eight species tested, suggesting that resistance-associated cell death might be a conserved immune mechanism of brown algae. We will report our progress on the molecular characterization of this response, such as the development of *in situ* labeling techniques or mining of the *Ectocarpus* genome for potential disease resistance genes.

485. Host adaptation as mechanism for speciation? Comparative analysis of *Sporisorium reilianum* isolates with different host preference

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Sporisorium reilianum is the causative agent of head smut of maize and sorghum. Haploid strains of different mating type fuse and form infectious dikaryotic hyphae that can penetrate young leaves. Although hyphae of *S. reilianum* can be found in all plant tissues, sporulation takes place exclusively in the inflorescence. We isolated haploid strains from spores of diseased maize and sorghum plants. Infection assays showed that maize isolates of *S. reilianum* (SRM) are infectious on maize but not on sorghum, while sorghum isolates of *S. reilianum* (SRS) are infectious on sorghum but not on maize. SRM and SRS strains are mating competent, and crosses lead to (a low rate of) spore formation on maize. Thus, the isolated SRM and SRS strains are varieties of the same species. Crosses of SRM and SRS strains do not cause sorghum disease and succumb to tissue-specific host defense. This suggests the existence of factors in SRM strains that inhibit virulence on sorghum. Segregation analysis indicated involvement of many genes in host adaptation. Using comparative genomics of maize and barley smut fungi we identified three genes with a potential inhibitory function for virulence on sorghum and supportive function for virulence on maize. Genome comparison of both varieties supported that SRM and SRS strains represent a species under evolution that develops into two distinct species because of adaptation to different hosts.

486. Functional analysis of the *velvetA* family in *Ustilago maydis*

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Ustilago maydis is a model fungus for the study of disease development in obligate plant pathogens. Members of the fungal-specific *velvetA (veA)* gene family are known to regulate sexual development as well as spore viability in *Aspergillus* sp., but the functions of these proteins have not been investigated in *U. maydis*. We predict that members of the *U. maydis veA* family will regulate sexual spore formation in plants, and thus have an effect on disease progression in planta. Three *U. maydis* genes, *Um04203, Um00893 and Um01146*, were identified by BLAST searches as orthologs of the *veA* family in *Aspergillus nidulans*. A modified version of the GATEWAY system was used to make gene deletion constructs for each *UmveA* gene. Transformed *U. maydis* colonies that grew on carboxin-amended medium were tested for gene replacement by polymerase chain reaction, and retested by Southern blot to confirm that the UmveA coding sequences had been deleted. To date, four *Um04203*$^{-}$, two *Um00893*$^{-}$ and two *Um01146*$^{-}$ mutants have been confirmed by Southern analysis. In preliminary virulence assays on maize, the *Um04203*$^{-}$ mutants were similar to wild-type, but the *Um00893*$^{-}$ mutants produced no galls or spores, suggesting that this gene is essential for disease development.

487. A cloning and characterization of DnaJ like homologue from *Cryphonectria parasitica*

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Differential display for the expression profiles of the wild type *Cryphonectria parasitica* and its virus-infected isogenic hypovirulent strain revealed several transcripts of interest, which show the difference in the accumulation of transcript and significant matches with fungal genes of known function. In addition, microarray assay exhibited that a molecular chaperone Hsp70 is specifically regulated by the presence of hypovirus while other representative genes in hest shock responsive pathway are not suggesting the specific but rather than general responsiveness. Thus, we further analyzed an amplified PCR product with the sequence similarity to the known fungal DnaJ-like gene, a molecular chaperone and regulator of Hsp 70, because first, differential display for the expression profiles of the wild type *Cryphonectria parasitica* and its virus-infected isogenic hypovirulent strain revealed the difference in the accumulation of transcript, secondly, microarray assay exhibited that a molecular chaperone Hsp70 is specifically regulated by the presence of hypovirus. The cloned DnaJ-like gene *Cpdj*1 product consists of 379 amino acids with a predicted molecular mass of 40.6 kDa and a pI of 7.79. Sequence comparisons revealed that the deduced protein sequence of the *Cpdj*1 gene exhibited a high homology to all the known DnaJ-like proteins with the highest homology to a DnaJ gene from *Neurospora crassa*, and showed the preservation of the conserved hall mark J-region. Disruption of the *Cpdj*1 gene resulted in slow growth and produced colonies characterized by little aerial mycelia and deep orange in color. Accordingly, reduced virulence of the *Cpdj*1-null mutant as compared to the wild type was observed, which can be ascribed to the growth defect. This reduced growth rate was magnified when the *Cpdj*1-null mutant was cultured under the heat-stress condition of 30°C instead of 25°C. Reduced conidiation was also observed from the *Cpdj*1-null mutant indicating that the *Cpdj*1 gene is not essential for cell viability but is required for the appropriate cellular process including normal growth rate and sporulation. Northern blot analysis showed that the accumulation of *Cpdj*1 transcript was increased as the culture proceeded i.e., the level of *Cpdj*1 transcription peaked at 5 day after incubation and remained thereafter. However, the strong induction was observed when the culture was subject to high temperature. Hypoviral hindrance of transcriptional induction was observed when the culture was placed in heat stress condition indicating the involvement of hypovirus in fungal response to heat stress.

488. Effector proteins of powdery mildew fungi

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The ascomycete *Blumeria graminis* f.sp. *hordei* (*Bgh*) is the causal agent of the powdery mildew disease on the monocot barley (*Hordeum vulgare*). Suppression of host defense responses and accommodation of the intracellular feeding structure (haustorium) are indispensable for this obligate biotrophic phytopathogen. To manipulate its host plant, *Bgh* uses small, secreted effector proteins. Candidate effectors were bioinformatically identified by presence of a predicted secretion signal. Functionality of the signal peptide was confirmed in a yeast-based genetic screen. Upon transient expression in barley a subset of these candidates increased the penetration efficiency of the fungus. Currently, we employ a yeast-two-hybrid assay to screen a cDNA library from *Bgh*-infected barley leaves to disclose host target proteins as well as fungal interacting proteins of the effector candidates. Real time PCR analysis revealed that transcript levels of two candidates are increased during appressorium and haustorium formation, respectively. For the former *Bgh* effector candidate we identified and cloned a homologous gene from a powdery mildew fungus (*Golovinomyces orontii*) that is virulent on the dicot reference species *Arabidopsis thaliana*. Stable expression of this gene in Arabidopsis and subsequent challenge with the adapted fungus might unveil conserved infection strategies for the fungus on monocots and dicots.

489. Alcohol fermentation and hypoxia adaptation in the pathogenic mold *Aspergillus fumigatus*

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Recent studies have shown that tolerance to hypoxia may be a virulence attribute of fungal pathogens. Currently, our knowledge of *Aspergillus fumigatus's* (*Af*) ability to survive in hypoxic conditions during fungal pathogenesis is limited. Using a murine model of invasive aspergillosis and ^{1}H-NMR metabolomics, we have found ethanol and lactate in the lungs of *Af* infected mice. This result suggests that *Af* utilizes fermentation pathways *in vivo* to cause disease. During ethanol fermentation, pyruvate, the end product of glycolysis, is metabolized by pyruvate decarboxylase (Pdc) to acetaldehyde, which is subsequently reduced to ethanol by alcohol dehydrogenase III (*alcC*). Under growth conditions containing glucose as a carbon source and low oxygen levels we observed that pyruvate decarboxylase activity in *Af* cell-free extracts is elevated. We identified 3 potential pyruvate decarboxylase genes (*pdcA*, *pdcB*, and *pdcC*) in *Af* and observed that all 3 *pdc* genes were induced under hypoxic conditions. Generation of null mutants in these genes and the alcohol dehydrogenase *alcC* has allowed us to determine the role of fermentation in hypoxia adaptation in this pathogenic mold. Our results indicate that PdcA is the pyruvate decarboxylase primarily responsible for alcohol fermentation in *Af*. The loss of *pdcA* results in a decrease of Pdc activity of ~80% and a reduction of ethanol production below the detection limit of 0.01%. Loss of *alcC* results in elimination of 95% of EtOH production. Surprisingly, we did not observe a growth defect of the *pdcA* or *alcC* mutant under hypoxic conditions. We hypothesize that either residual Pdc activity from PdcC or reduction of pyruvate to lactate by lactate dehydrogenase is enough to allow survival under hypoxic conditions. We further hypothesize that either the elimination of all *pdc* genes or a *pdcA pdcC* double knockout will affect the ability of *Af* to survive under hypoxic conditions and cause disease.

490. Meadow fescue response to mutualistic and parasitic growth of its endophyte, *Epichloë festucae*

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Epichloë festucae is a mutualistic fungal symbiont of cool season grasses, that produces bioprotective alkaloids and can improve the drought stress tolerance of its host. It colonizes endophytically all above ground plant organs, and displays a dual transmission mode. In some reproductive tillers it retains its benign endophytic growth and is seed transmitted, on others it produces an external ascogenous hyphal layer (stromata) that chokes the inflorescence. To study host responses of meadow fescue (*Lolium pratense*) on benign and pathogenic fungal growth, we sequenced 112,000 ESTs from normalized cDNA libraries, made from stromata (S), and endophyte- infected symptomless inflorescences (I). Fungal reads were removed by mapping them against the genome of *E. festucae*, and based on blastx matches to the NCBI protein database. The plant dataset assembled into 20,840 unigenes, 70 % of which matched a plant gene in the NCBI db. Gene expression was analyzed by 454- pyrosequencing of non-normalized cDNA from S and I tissues, then mapping the reads to the assembled plant unigene dataset. We identified 229 unigenes upregulated in S, and 177 unigenes upregulated in I (at levels ≥ 5x). Annotations of plant genes most highly upregulated in S suggested their involvement in repression of flowering, stress responses, and signal transduction. In benignly-infected inflorescences, genes for putatively antifungal proteins were among those most highly upregulated.

491. Molecular analysis of the host-pathogen interaction between *Agaricus bisporus* and *Verticillium fungicola*

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'Dry bubble' disease of the white button mushroom Agaricus bisporus results from infections with Verticillium fungicola. Recent advances in Agaricus and Verticillium molecular technologies provide opportunities to dissect the infection processes and to gain an understanding of the genetic, biochemical and physiological processes that regulate the interaction between the fungal host and its mycopathogen. We have exploited a combination of approaches to characterise the fungal-fungal interaction. SSH and cDNA libraries of A. bisporus infected with V. fungicola were used to identify pools of host and pathogen response genes, up- or down-regulated during development of disease lesions (cap spotting). Bioinformatic analysis revealed a range of response genes with various biological functions: biotic and abiotic stress; signalling; protein synthesis; cell wall structure and function. To expedite functional analysis of disease response genes, we have developed RNAi hairpin-mediated gene suppression for A. bisporus. Specific mushroom genes identified from SSH-cDNA libraries and belonging to different functional groups have been down-regulated and characterised through infection trials and quantitative PCR approaches.

492. The function and regulation of a polygalacturonase gene *Mfpg1* in *Monilinia fructicola* pathogenesis.

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A 1-kb genomic DNA fragment from the brown rot pathogen *Monilinia fructicola* was cloned. Sequence analysis revealed an open reading frame of 1095 nucleotides encoding 365 amino acids with a signal peptide of 21 aa. The deduced amino acid sequences showed 83% identity to those of endopolygalacturonse genes of *Botrytis cinerea Bcpg1* and *Sclerotinia sclerotorium sspg1d*. The predicted mature protein carries a polygalacturonase functional domain at C-terminal. Thus the gene was named *Mfpg1* and is the first endopolygalacturonase gene isolated and characterized in *M. fructicola*. Southern analysis demonstrated that this gene presents as a single copy. About 4.6 kb of *Mfpg1* flanking regions were further isolated by inverse PCR and the promoter region with *Mfpg1* ORF was fused to *gusA* to produce an MFPG1-GUS fusion protein. A vector carries *Mfpg1* gene replacement construct with 4.5 kb *Mfpg1* homology was also created. Based on the GUS activity expression, *Mfpg1* was expressed in the early stage of infection on peach petals and also on infected fruits. Further characterization of *Mfpg1* function and regulation will be presented.

493. Ftf1 is a multi-copy gene that encodes a transcription factor specifically involved in virulence in *Fusarium oxysporum*

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We have described a new transcription factor (ftf1), with potential to be a virulence factor in *Fusarium oxysporum*, as it is found only in highly virulent strains and is drastically upregulated during early stages of infection of the host plant. We have also identified a highly homologous gene to ftf1, named ftf2, which can be found in pathogenic and non pathogenic fungi. Experimental results obtained in *F. oxysporum f.sp. phaseoli* and analyses of the genome sequences of other *Fusarium sp.*, show that ftf1 is a multi-copy gene only present in *F. oxysporum*. The four copies in F. oxysporum f.sp. phaseoli are located in a small chromosome, closely linked to copies of transposon marsu, while the nine copies found in the genome of the lycopersicy strain are scattered over different chromosomes of the optic map, but also linked to different kinds of transposons, including marsu and Fot types.
A gene silencing strategy was followed to demonstrate that ftf1 is required for virulence. *Agrobacterium tumefaciens*-mediated transfer of a hairpin-expression vector carrying fragments of the ftf1 gene allowed efficient silencing of ftf1 expression in highly virulent strains of *F. oxysporum f.sp. lycopersici* and *f.sp. phaseoli*. Several independent silenced transformants were tested for pathogenicity and virulence in inoculation assays using tomato and common bean as host plants. Inoculated plants showed clear symptoms of vascular disease but, in tomato, growth and size were similar to the non-inoculated controls, and, in common bean, the disease progression was similar to that induced by weakly virulent strains. These results demonstrate that silencing of ftf1 do no abolish pathogenicity but reduces virulence against the host plant.
The high homology between the genes ftf1 and ftf2 but their different role in the infection of the host plant, poses questions on gene evolution that may be of interest in unraveling how pathogenic strains have evolved from non pathogenic ones and on the origin of the formae speciales in *F. oxysporum*.

494. Analysis of gene expression and antibiotic production in biocontrol by Trichoderma spp.

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Trichoderma species are mycoparasites of phytopathogenic fungi. Upon recognition of a host they form specialized structures, secrete a complex set of cell wall degrading enzymes, and produce antibiotics. Therefore, during the interaction with the host there are important changes in gene expression. We have analyzed changes in gene expression in the interaction with different hosts in search for host specific genes by direct counting of cDNA sequences. Our analysis suggests that there are, indeed, host specific responses. In addition, we have generated mutants in the cfwA gene of T. virens, which are affected in the production of non-ribosomal peptides and polyketides. This approach allowed us to identify novel antibiotics produced by T. virens. In order to determine the relevance of such secondary metabolites for plant protection we analyzed the behavior of the mutants both in vitro and in vivo. The mutants clearly overgrow the host but are incapable of killing it, even in vitro, and are affected in their capacity to promote growth of tomato seedlings.

495. The brown algal pathogen *Eurychasma dicksonii*: A model oomycete to study the evolution of pathogenicity

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The intracellular, obligate-biotrophic pathogen *Eurychasma dicksonii* is the most widespread eukaryotic pathogen of marine brown algae, and also the most basal member of the oomycete lineage. This algal parasite has the broadest host range described so far for marine pathogens and occurs worldwide in cold and temperate waters. Currently, nothing is known about the molecular mechanisms that determine the pathogenicity of this generalist parasite. We have developed a Real-Time PCR assay that reliably quantifies *Eurychasma* infection in brown algae and found that various clonal *Ectocarpus* strains show differential susceptibility towards the oomycete pathogen. Established on laboratory cultures this assay is also applicable for the detection of the pathogen in natural brown algal populations (1). Due to its availability in culture, its phylogenetic position and the recently completed sequencing of the genome of one of its brown algal hosts, *Ectocarpus siliculosus*, we are currently using *Eurychasma dickosnii* as a model organism to study the evolution of pathogenicity among oomycetes and chromealveolates.
(1) Gachon, CMM, Strittmatter M, Müller DG, Kleinteich J and FC Küpper (in press). Detection of Differential Host Susceptibility to the Marine Oomycete Pathogen *Eurychasma dicksonii* by Real-Time PCR: Not All Algae Are Equal. *AEM*

496. *Agrobacterium tumefaciens* mediated transformation of *Phoma tracheiphila*, the mal secco disease casual agent of citrus

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Mal secco disease of citrus is one of the most devastating diseases of susceptible citrus and lemons in particular. Although the disease is restricted to the Mediterranean basin it is of a major concern to all citrus growing and importing countries in the world. The disease is caused by the pathogenic fungus *P. tracheiphila* (Petri) Kanc. & Gik. The diseases epidemiology, toxins involved in the fungus pathogenicity and its identification by classical and molecular methods have been studied and published. Molecular mechanisms, genes involved in pathogenicity and the molecular host pathogen interaction were less studied. In order to understand these molecular relations between the fungus and its host, a method for the identification of genes involved in pathogenicity is needed. This work describes a method for efficient transformation of *P. tracheiphila*'s spores (phialoconideia) by the *A. tumefaciens* mediated transformation method and the generation of mutated *P. tracheiphila* isolates, displaying reduced pathogenicity on Rough lemon seedlings. This study also describes the development of a quick, repetitive, and reliable method for the screening of *P. tracheiphila* mutants on Rough lemon seedlings. This is the first report of *A. tumefaciens* mediated transformation of *P. tracheiphila* and screening of the fungal mutants for the isolation of nonpathogenic mutants of the pathogenic fungus. Results will be presented.

497. Impact of homocitrate synthase on *Aspergillus fumigatus* pathogenesis

Felicitas Schöbel and Matthias Brock

Fungi, such as *A. fumigatus*, are able to synthesize lysine de novo via the alpha- aminoadipate pathway. In contrast, lysine is an essential amino acid for humans and must be obtained from the diet. Therefore, enzymes of this pathway might represent potential targets for new antifungals. However, until now it is unclear, whether *A. fumigatus* can satisfy its need for lysine from the degradation of the surrounding host tissue, e.g. from the degradation of proteins. This assumption is supported by the virulence attenuation of an *A. fumigatus* methylcitrate synthase mutant in murine infection models (Ibrahim-Granet et al 2008). Such a mutant accumulates toxic amounts of propionyl-CoA, which most likely derive from the degradation of proteins during pathogenesis. Our major interest was to verify, whether the de novo synthesis of lysine is only essential for the onset of an invasive aspergillosis or also during later stages of infection. To prove this assumptions, we deleted the homocitrate synthase, the first enzyme of the alpha-aminoadipate pathway, from the genome of *A. fumigatus*. The mutant revealed that the de novo lysine biosynthesis plays a critical role for conidia germination on unhydrolysed proteins. In vivo studies confirmed an importance of lysine biosynthesis especially during the onset of infection, whereas the phenotype was partially complemented by feeding mice with elevated levels of lysine.

Ibrahim-Granet O., et al. (2008) Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. Cell Microbiol.; 10(1):134-48.

498. Potent inducers of deoxynivalenol production by *Fusarium graminearum*

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Fusarium head blight of wheat, caused by *F. graminearum*, is one of the most important diseases of wheat not only because of yield losses but also the contamination of grain with trichothecene toxins such as deoxynivalenol (DON). As well as playing an important role in the virulence of the pathogen, DON has severe health consequences for humans and animals which consume contaminated grain. An intriguing aspect of the pathogen's biology is that the production of DON occurs at much higher levels during the infection process than during axenic culture, even on plant-derived media such as autoclaved grain. Presumably, the fungus produces toxins in response to unknown signals of plant origin. We developed a reporter strain of *F. graminearum* carrying a *TRI*-gene promoter linked to the green fluorescent protein gene and used this strain in a high-throughput screen to identify compounds that induce high levels of DON production in culture. Through this system, we have identified a number of compounds that induced the genes involved in the biosynthesis of DON to levels equivalent to those observed during infection, and resulted in extremely high concentrations (>1500 ppm) of DON being produced in culture filtrate after seven days of culture. Some of these compounds are naturally present in wheat and increase in concentration in heads following inoculation suggesting that they may act as *in planta* DON inducers. In addition, *F. graminearum* genes co-regulated under high DON-inducing culture conditions have been studied using Affymetrix expression profiling and other co-regulated fungal genes and gene clusters identified. Together, these studies open the way to reduce the production of DON during infection process by either genetic or chemical modulation of the production and/or action of host inducers.

499. Quantitative proteomic analysis of two different strains of *Stagonospora nodorum* using isobaric Tags for Relative and Absolute Quantification (iTRAQ).

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A study of the relative differences in protein abundance between two strains of the wheat pathogen *Stagonospora nodorum* was undertaken utilising the iTRAQ system, a robust and reproducible method for whole proteome quantitative analysis. Proteins from SN15 (wildtype) and *gna1-35* (G-alpha mutant) were digested with trypsin and labeled with iTRAQ reagents 114 m/z and 116 m/z, respectively, in triplicate. The peptides were separated by a combination of strong cation exchange and reverse phase chromatography followed by detection on a 4800 MALDI-TOF/TOF mass spectrometer. Analyses of MSMS data were performed by searching against the translated *Stagonospora nodorum* genomic database with three replicates analysed independently and then collectively to allow assessment of reproducibility for the methods developed. Over 1200 proteins were identified with > 95% confidence, ~8% of which were differentially expressed in the *gna1-35* strain. These results provide an initial analysis of the proteome of *Stagonospora nodorum* with implications for the assessment of the accuracy of the genome annotation and treatment of this pathogenic fungus.

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500. Effector evolution in the *Hyaloperonospora arabidopsidis* species complex.

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Hyaloperonospora arabidopsidis parasitic to *Arabidopsis thaliana* (previously referred to as *Peronospora parasitica*) is a model system for dissecting the interactions between obligate biotrophic parasites and plants. Although host jumps are usually frequent in biotrophic pathogens, little is known about the impact of host jumps on the genome and effectorome of the respective organisms. Within the *Hyaloperonospora arabidopsidis* cluster, several host jumps can be observed in closely related species, e.g. to *Draba*, *Microthlaspi* and *Reseda* (Resedaceae). These offer perfect models for investigating the evolutionary fate of effector genes. Through PCR and transcriptome analyses we could identify several effectors in *Hyaloperonospora erophilae* and *Hyaloperonospora thlaspeos-perfoliati* that are under positive selection, while others are undergoing rapid pseudogenization. In the ATR13 homolog of *Hyaloperonospora erophilae*, the RXLR motif that is essential for targeting the host cytoplasm has been altered, thus shutting down the delivery of this effector. This provides a first evidence that effectors that are recognized by the host plants as being alien or that do not fit their target anymore are quickly eliminated during micro- and mesoevolution.

501. Chorismate synthase and colonization of xylem vessels of *B. napus* by the phytopathogenic fungus *V. longisporum*,

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Verticillium longisporum is a devastating soil-borne fungal pathogen of oilseed rape (*Brassica napus*). The fungus colonizes xylem vessels of host plants. The nutritional status of the fungus in xylem vessels is not yet known. The xylem sap is a water solution containing low concentrations of proteins, sugars and in addition amino acids. The extent of the plant defense mechanisms in the xylem and the subsequent responses of the fungus are largely unexplored. We analyzed whether biotrophic fungal growth depends on intact aromatic amino acid biosynthesis or is supported by the amino acids provided by the plant xylem. Therefore, we constructed bradytrophic mutants of *V. longisporum* impaired in aromatic amino acid biosynthesis. We knocked down ARO2, the gene for chorismate synthase by RNAi technology. The ARO2 encoded enzyme catalyzes the synthesis of chorismate, the precursor of the three aromatic amino acids. The resulting delta*aro2* bradytrophs showed no inhibition during saprophytic growth on minimal medium. In contrast, their virulence in *B. napus* was markedly reduced. Therefore, silenced mutants were able to produce enough chorismate and aromatic amino acids to sustain growth. It is tempting to speculate that additional aromatic compounds deriving from chorismate might be required for the biotrophic life. These might be secondary metabolites required for communication or defense.

502. *SGE1*, a putative transcriptional regulator is required for pathogenicity in *Fusarium oxysporum* f. sp. *lycopersici* and regulates infection phase specific genes.

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Fusarium oxysporum f. sp. *lycopersici* is a soil-born fungus that causes vascular wilt disease in tomato by penetrating the plant roots and colonizing the vascular tissue. Previously, we generated 10,290 random insertion mutants in order to identify genes involved in pathogenicity using T-DNA of *Agrobacterium tumefaciens* as an insertional mutagen. One of the pathogenicity mutants identified in this study was found to contain a single copy T-DNA integration in a gene, designated *SGE1*, which shows similarity to the morphological switch regulators *Candida albicans WOR1* and *Histoplasma capsulatum RYP1*. Gene knock-out and complementation studies confirmed that *SGE1* is required for pathogenicity. In addition, microscopic analysis revealed that 1) Sge1 is localized in the nucleus and 2) Sge1 is not required for root colonization and penetration but is required for *in planta* growth and for expression of genes specifically upregulated during infection. We speculate that Sge1 is required in plant-pathogenic fungi for a morphological switch from a saprophytic to an infectious growth phase.

503. Relative importance of different high affinity iron uptake systems for virulence of *Cochliobolus heterostrophus*

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Iron is an essential micronutrient for nearly every organism on earth, including plant and animal pathogens. Host iron is bound to ferritin or other chelators, so successful pathogens must possess a method for high affinity iron acquisition to liberate it. Fungi have two known mechanisms for this. Many produce small Fe^{3+} chelating peptides called siderophores, which can be involved in iron acquisition or homeostasis. Fungi can also reduce Fe^{3+} at the cell membrane and transport it with a high affinity iron permease (FTR), a process called reductive iron assimilation (RIA). The maize pathogen *Cochliobolus heterostrophus* possesses both siderophore (produced by the nonribosomal peptide synthetase NPS6) and RIA mediated iron acquisition and both are iron regulated, suggesting involvement in iron homeostasis. *nps6* mutants display reduced virulence and increased sensitivity to low iron and oxidative stress. To test whether loss of RIA also affects virulence and sensitivity to low iron and oxidative stress, an *ftr*>-deletion strain was generated. Unlike *nps6*-deletion strains, *ftr* strains are like WT with respect to these characteristics. *nps6;ftr* double mutants, generated by crossing *nps6* to *ftr* mutants, display growth defects on complete medium compared to *nps6*, as well as a reduction in conidiation. Testing these mutants for sensitivity to low iron, oxidative stress, and virulence will indicate the relative importance of RIA *vs* siderophores for *C. heterostrophus*. If the double mutant can still grow *in planta*, it will indicate that the fungus has a novel method for iron acquisition.

504. Withdrawn

505. Differential localization of putative effector proteins by the blast fungus *Magnaporthe oryzae* during biotrophic invasion

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Magnaporthe oryzae is a hypervariable fungus able to defeat introduced resistance *(R)* genes within 1 or 2 years after their deployment. The initial biotrophic infection stage requires the fungus to secrete effector proteins inside the rice cell to control plant processes. Secreted effector proteins are presumed to play a critical role in disease establishment. Understanding this process will contribute significantly towards disease control. However, the effector secretion mechanisms used by any filamentous pathogenic fungi still remain unknown. Analysis of chimeric proteins produced by fusing avirulence proteins to the green fluorescent protein (GFP) showed that they were secreted by IH and accumulated in a distinct structure, Biotrophic Interfacial Complex (BIC), located between the IH cell wall and the extra-invasive-hyphal membrane (EIHM). We extend this analysis by examining secretion of additional putative effectors identified by microarray analysis. For each of four putative effectors, we fused a fluorescent reporter, enhanced yellow fluorescent protein (EYFP) or monomeric red fluorescent protein (mRFP) at the C- terminus of the entire protein coding sequence and expressed the fusion protein using the native promoter. Using live cell imaging we visualized secretion of these chimeric proteins in planta. Our results show that all 4 proteins are specifically expressed during biotrophic invasion. They exhibited different patterns of secretion ranging from colocalization with AVR-Pita:GFP at the BIC to intense uniform distribution within the EIHM around the entire IH. Understanding how these putative fungal effector proteins function in host infection will represent a major advance in molecular plant-pathogen interactions.

506. Cell-entry motifs of effectors from three eukaryotic kingdoms bind a common receptor family

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Bacterial effectors have been shown to enter host cells via the type three secretion system. There is no evidence of anything resembling a type three secretion system in oomycetes or in any other eukaryotes. The N-terminal RXLR-dEER motif from P. sojae effector Avr1b can translocate proteins including GFP into soybean root cells without any pathogen encoded machinery. The N-terminal RXLR-dEER motif of Avr3a from P. infestans has been shown to translocate beta-glucuronidase and the C-terminus of Avr3a into host cells. Effectors from the malaria parasite, Plasmodium utilize an N-terminus PEXEL (RXLXE/D/Q) motif to enter host cells. The oomycete and Plasmodium protein translocation motifs have been shown to be interchangeable amongst the two pathogens. We have shown that fungal effectors contain variant RXLR-dEER motifs that can also translocate proteins into plant cells. We have identified a family of candidate receptors that bind to RXLR-dEER-like motifs from all three kingdoms of eukaryotic pathogens. The identity and location of the receptors suggest that all these effectors enter cells via receptor-mediated endocytosis.

507. Rsp3, a repetitive secreted protein, is required for pathogenic development in the smut fungus *Ustilago maydis*.

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On the plant surface, the dimorphic fungus *Ustilago maydis* switches from yeast to hyphal growth and develops un-melanized appressorium required for plant infection. In an attempt to identify genes involved in appressorium function we performed transcriptional profiling of the solopathogenic strains SG200 (infective) and SG200Kpp6$^{\Delta EF}$ (no infective) grown on the plant surface. This analysis identified *rsp3* as a gene whose expression is 5-fold higher in SG200 than SG200Kpp6$^{\Delta EF}$. *rsp3* (repetitive secreted protein 3) encodes a putative secreted protein containing several repetitive domains. Rsp3 is a variable protein and the repeats units differ in field isolates of *U. maydis*. The N-terminal half of the protein is highly conserved between *U. maydis* and its close relative *Sporisorium relianum*, however the C-terminal domain which contains the repeats shows a large number of differences. *rsp3* is highly expressed in appressorial stage and during subsequent stages of the biotrophic development. To find out the role of *rsp3*, deletion mutants were generated in the solopathogenic strain SG200. In axenic culture, SG200-delta rsp3 was delayed in filament formation and was highly susceptible to cell wall disturbing compounds, suggesting a putative role in cell wall integrity. When maize plants were infected with SG200-delta rsp3 strains we observed that the mutants produced functional appressoria but were affected in subsequent stages of development. Only occasionally small tumors were induced in the infected leaves but anthocyanin production was absent and necrosis in the infected plant tissue was induced. During intracellular growth SG200-delta rsp3 produced short hyphae and numerous swollen branches. We will discuss the role of rsp3 during biotrophic development of *U. maydis*.

508. Crazy ants: the fine detailed manipulation and exploitation of ants by the fungus *Ophiocordyceps (=Cordyceps) unilateralis*

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The fungus *Ophiocordyceps (=Cordyceps) unilateralis* manipulates ant behavior. In Thai rainforests infected Carpenter ants (*Camponotus leonardi*) leave their colony in the high canopy and descend 20m to the forest floor. Infected ants find and bite onto the underside of leaves. They preferentially choose leaves within a narrow band 25cm off the ground and on the North side of the plant. The timing of biting is highly synchronized around noon. Ants die a few hours after biting the leaf. Dead ants remain attached to the leaf for a year and in some cases 18 months. Unlike other entomopathogens *O. unilateralis* has evolved a sophisticated storage strategy and division of labor that facilitates such a long term strategy. The mechanism of behavioral change is targeted destruction of a number of mandible muscle organelles: myofibrils, mitochondria and sarcoplasmic reticula. Ants bite leaves because the fungus induces lockjaw. The sophisticated adaptations by this fungus to manipulate and exploit its living and dead host challenges our current model of fungal- insect parasite systems. We are developing a functional genomic approach, that includes de novo genome sequencing, to explore the proximate mechanisms of behavioral change in greater detail.

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509. Unravelling the mechanism of RxLR mediated translocation of Oomycete effector proteins

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Most oomycete pathogens invade their hosts in a biotrophic manner, which means that they try to avoid host recognition and/or suppress host immune responses. Several biotrophic pathogens translocate effector-proteins into their host cells, which help to establish a successful infection. Oomycete pathogens do not possess a type III secretion machinery as bacteria have, and instead they seem to have developed a different effector translocation system. They are able to translocate proteins that contain an RxLR-EER motif located after the signal peptide. It was shown that this motif is important for effector translocation as mutating this domain stops translocation into the host cells. The mechanism by which oomycetes direct their RxLR-EER effectors into host cells is as yet unknown and is the main focus of our research. It has been postulated that endocytosis processes or protein transporters are responsible. Here we present our latest results, which give insight into the mechanism of the oomycete RxLR-EER protein translocation system.

510. Functional analysis of Phytophthora infestans RXLR effectors Avr2 and Avr3a

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AVR3a, the first effector characterized from Phytophthora infestans, contains an N- terminal RxLR and dEER motif required for transport across the host plasma membrane. Genomic resources have revealed approximately 500 rapidly diverging RXLR effectors. Transient expression of effectors in collections of cultivated and wild Solanum species identifies sources of resistance (R) genes that recognise specific effectors. By silencing effectors in P. infestans we identified >15 functionally essential and >10 redundant for virulence. To examine effector functions we are identifying interacting plant host proteins. Subcellular localisations of effectors and targets are being uncovered using fluorescent labels. We will present our progress in the investigation of pathogenicity functions of the AVR2 and AVR3a avirulence effectors. We will present data visualizing translocation from haustoria and showing that these effectors interact with different host proteins to establish infection. Approaches such as virus-induced genes silencing, are being used to determine the roles of host targets in defence.

511. Identification of plant proteins targeted by oomycete RXLR effectors using in planta co-immunoprecipitation

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Oomycete plant pathogens secrete a class of so called RXLR effectors that are translocated inside the plant cells to establish infection. These effectors may function by targeting the plant proteins through physical interactions which may alter the functions of the targeted proteins resulting in a favourable outcome for the pathogen. We aim at identifying the plant proteins targeted by oomycete effectors and the affected physiological processes. We selected 52 validated oomycete RXLR effectors and homologs for this study. We made expression constructs based on high-expression vector pJL-TRBO, a binary plasmid containing a modified *Tobacco mosaic virus* with its coat protein gene replaced by cDNAs coding for FLAG-tagged mature oomycete effectors. Effector constructs were delivered into the leaves of *Nicotiana benthamiana* by agroinfiltration and effectors were expressed under the control of the viral coat protein promoter. The leaves were harvested 2-3 days after infiltration and total proteins were extracted. Effector proteins and their interactors from the plant were co- immunoprecipitated (co-IP) with anti-FLAG resins under non-denaturing conditions. Bound proteins were specifically eluted using 3X FLAG peptides, separated by SDS- PAGE and visualized by colloidal Coomassie blue staining. Protein bands were excised, digested with trypsin, and identified by LC-MS/MS peptide ion spectrum matching. So far we have expressed over 30 effectors to sufficient levels for co-IP and subsequent MS identification of precipitated proteins. We will report and discuss identified effector target proteins, and any alterations in plant immunity resulted from overexpression or virus- induced gene silencing of these targets. The interactions will also be confirmed by alternative approaches such as reverse Co-IP, yeast two hybrid or BiFC techniques.

512. The transcription factor FgStuA influences spore development, pathogenicity and secondary metabolite production in *Fusarium graminearum*.

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Members of the APSES family of fungal proteins have been identified as key regulators of fungal development, controlling processes such as mating, sporulation and dimorphic growth. We deleted the *FgStuA* gene in *Fusarium graminearum* and show that the mutant is greatly impaired in spore development, pathogenicity and secondary metabolism. *FgStuA* is closely related to *FoStuA* in *F. oxysporum*, but unlike *FoStuA* mutants the *FgStuA* mutants were greatly reduced in pathogenicity both on wheat and apple slices. The lack of ability to cause disease on wheat heads may be due to lack of trichothecene accumulation *in planta*. The *FgStuA* mutant also had a white/yellow mycelial phenotype compared to the red pigmented (aurofusarin) wild-type, had reduced aerial mycelium, susceptibility to oxidative stress, and had a less hydrophobic surface. Microarray analysis showed that most phenotypes could be inferred from gene expression data, such as down-regulation of the trichothecene gene cluster in the mutant. In an attempt to separate primary and secondary effects of *FgStuA* deletion, we carefully examined gene expression data together with promoter analysis and comparative genomics. The genes flanking *FgStuA* are conserved and syntenous in other fungal genomes and contain a gene encoding a putative clock controlled protein. FgStuAp and other APSES proteins share significant homology with DNA-binding domains of transcription factors controlling the critical G1/S phase cell cycle transition in both *S. cerevisiae* and *S. pombe*. Genes within MIPS Functional Category (FunCat) 10 "Cell cycle and DNA processing" are enriched among those more highly expressed in the *FgStuA* mutant than wild-type. *Aspergillus* StuAp response elements (A/TCGCGT/ANA/C) also were found highly enriched in promoter sequences for FunCat 10 genes, compared to the genome as a whole. Our results suggests that FgStuAp may act primarily as a repressor involved in cell cycle regulation, and may act only secondarily on sporulation, pathogenicity, and secondary metabolism.

513. A genetic linkage map of *Mycosphaerella fijiensis*, using SSR and DArT markers

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Mycosphaerella fijiensis is the causal agent of black leaf streak or Black Sigatoka disease in bananas. This pathogen threatens global banana production as the main export Cavendish cultivars are highly susceptible. Previously a genetic linkage map was generated predominantly using anonymous AFLP markers. To assist genome assembly and the selection of markers for population analysis we generated a genetic linkage map of *M. fijiensis* using 87 SSR (Simple-Sequence Repeat) markers, 3 VNTR (Variable Number of Tandem Repeat) markers, the mating type (*Mat*) locus and 235 DArT (Diversity Arrays Technology) markers. The segregation of these markers was studied in 136 individuals derived form a cross between CIRAD086 (Cameroon, (*Mat*1-1) x CIRAD 139A (Colombia, *Mat*1-2). The genetic linkage map comprises 19 linkage groups covering 1417 cM. The arrays containing individual fragments of the genomic representation of *M. fijiensis* generated DArT markers with a 90% genotype call rate and 98.8% reliability score. In total, 87% of the markers could be positioned reliably with high LOD scores (LOD >10). Due to the excellent genome coverage and high quality we decided to sequence the DArT markers to align this genetic map with the genome sequence of CIRAD086, which will considerably contribute to the current genome assembly (http://genome.jgi- psf.org/Mycfi1/Mycfi1.home.html) of this important fungus.

514. DNA homologous recombinational repair genes are involved in growth and pathogenicity of *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, the most important disease of rice. DNA recombination is reported as an important factor participating in the variability of *M. grisea*. In order to clarify the role of DNA recombination in pathogenesis and variability, *Rhm51*, *Rhm52* and *Rhm54*, homologs for *S. cerevisiae RAD51*, *RAD52* and *RAD54*, respectively were cloned. Northern blot analysis revealed that these genes are highly induced by Methyl methanesulfonate (MMS) and to some extent by Methyl Viologen (MV). The genes are also constitutively expressed at low levels during the cell cycle. GFP reporter revealed that *Rhm54* is expressed in invasive hyphae *in planta*. Disruption mutants of *Rhm51* and *Rhm54* were constructed. These mutants showed reduced growth and were highly sensitive to MMS and hydrogen peroxide. Although spores from both mutants and wild type do germinate and form appressoria, *rhm51* and *rhm54* mutants have a reduction in sporulation capacity compared to wild type. Inoculation assay revealed that the number of lesions fomed on comatible rice cultivar was reduced in *rhm54* mutants. These results indicate that DNA homologous recombinational repair genes are involved in growth and pathogenicity of *M. oryzae*.

515. The calcium channels Mid1 and Cch1 in the plant pathogen *Claviceps purpurea*

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The aim of our work is to reveal the molecular pathways involved in polarized growth of the phytopathogenic ascomycete *Claviceps purpurea*. The fungus penetrates the cuticle of stigmatic hairs of its poacean hostplants, grows down the style and through the ovarian tissue. The fungus grows mainly intercellularly and therefore decomposes the middle lamella between the plant cells. It is known that growing fungal hyphae possess a tip high calcium gradient just as other tip growing cells like pollen tubes, axons and others do. Using GFP based calcium sensors like Cameleon we want to image calcium dynamics in vivo. We also want to address the question how the tip high calcium gradient is established. It is conceivable that the fungus follows the "calcium trail" that is set up by the degradation of pectin during growth. For this purpose we have to postulate calcium channels located in the tip region of the hyphae. We cloned a homologue of the yeast stretch-activated, nonselective cation channel Mid1 and generated a knock-out mutant. In the delta*cpmid1* mutant mycelial growth is significantly slower than in the wildtype. Interestingly the delta*cpmid1* mutant is unable to penetrate and infect its host *Secale cereale*. The deletion mutant shows massive aggregations of cell wall material which indicates that the cell wall synthesis is affected by Mid1 function. It was shown before that in yeast mid1 interacts with the voltage-gated calcium channel Cch1 (Fischer *et al.* (1997), *FEBS Lett* **419**: 259-262). We recently identified the *Claviceps* homologue of this gene. Its functional analysis is under way.

516. Elucidating the function of a Forkhead transcription factor in *Ustilago maydis*

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We have identified a gene with similarities to forkhead proteins (Fkh1) that is required for pathogenic development of *Ustilago maydis*. Deletion strains of *fkh1* are able to form dikaryotic filaments that subsequently penetrate the plant, however, tumour development is impaired and spore formation is completely blocked. Chlorazole Black E staining identified a thick film-like substance accumulating around hyphae of *Delta-fkh1* strains that might be the result of a defense reaction of the host plant. In addition, *Delta-fkh1* mutant strains aggregate around the vascular bundles when compared to their respective wild-type strains. To identify Fkh1 putative target genes, arrays were conducted on plants infected with *Delta-fkh1*FB1 x *Delta-fkh1*FB2 (5 dpi). Array analysis revealed 245 differentially regulated genes. The list predominantly consists of genes encoding proteins in the following categories: metabolism, proteins with binding function or cofactor requirement, cellular transport and the largest category, unclassified proteins, which consists of many in planta regulated genes predicted to encode secreted proteins. Three of these unclassified secreted proteins were separately deleted based on their level of down-regulation observed in the array analysis. Each one of these mutants exhibited reduced levels of virulence at 7 and 14 dpi.

517. DNA non-homologous end joining pathway is varied among Japanese isolates of *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast, the most important disease of rice. DNA recombination is reported as an important factor participating in the variability in *M. oryzae*. In eukaryotes, two major systems for DNA recombination, homologous recombination and non-homologous end-joining (NHEJ) are known. Well- known NHEJ pathway involves Ku70/Ku80 heterodimer and DNA ligase IV. In order to evaluate the importance of these genes in the growth and pathogenicity of *M. oryzae*, deletion mutants of these genes were obtained from Japanese isolates Ina168 and Ina86-137. In Ina86-137, *mgku70*, *mgku80*, *mgku70/mgku80* mutants were significantly sensitive to methyl methanesulfonate (MMS) than the wild-type isolate. In Ina168, however, the sensitivity of all mutants to MMS was not significant. Gene targeting frequency of these mutants were assessed by *AdeA* gene deletion. All the mutants of Ina86-137 showed the elevation of targeting frequency to more than 80%, but Ina168 mutants did not show any significant elevation of the targeting frequency. These results indicated that *M. oryzae* has multiple pathways of NHEJ, one of those requires Mgku70/Mgku80 heterodimer and the others do not, and the contribution balance between pathways is different among strains.

518. Deoxynivalenol gene expression during wheat head infection by *Fusarium graminearum*

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The trichothecene mycotoxin deoxynivalenol (DON) is produced by several *Fusarium* species during infection of grain crops. DON acts as a virulence factor in wheat; its role in virulence in other hosts is variable. *Tri5* encodes trichodiene synthase, necessary for DON production. Previous experiments, including Affymetrix GeneChip assays, indicated that *Tri5* is highly expressed during wheat infection. Notably, *Tri5* expression appears to be correlated with the infection front, and to diminish once the fungus is fully established. We dissected wheat heads for several days following inoculation with *Fusarium graminearum* and monitored *Tri5* expression using quantitative reverse transcript PCR (qRT-PCR). qRT-PCR results will be presented which provide a thorough breakdown of *Tri5* gene expression during infection.

519. Small non coding RNAs in *Phytophthora infestans*

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Phytophthora infestans is an oomycete that causes late blight disease in potato. It has huge genome in comparison to other *Phytophthora* species. Genome sizes of *P .infestans* , *P. sojae* , *P. ramorum* are 237 MB, 95 MB, 65 MB respectively. The enormous genome size of *P. infestans* is attributed to regions of repeats and retrotransposons. In other organisms retrotransposons are controlled by small RNAs. Indeed, our data on detection of sRNA specific to several retrotransposons suggest that *P.infestans* employs sRNAs and gene silencing extensively to regulate these parts and probably other parts as well. To explore this possibility, deep sequencing (Solid sequencing platform) of small non coding RNAs of two isolates belonging to A1 and A2 mating types was carried out. From the sequencing data obtained we aim to identify siRNAs arising from silencing of transposons, avirulence and pathogenicity genes, mating type-specific genes, other lifecycle stage-specific genes, and miRNAs. We will determine the exact size of the sRNA specific to retrotransposons and also determine if siRNAs are priming generation of secondary siRNA to silence genes adjacent to transposons. There is accumulating evidence that some oomycete avirulence effector genes may be transcriptionally inactivated, possibly via silencing, to avoid recognition by host plants. *P. infestans*, and other oomycetes, have large numbers of effector genes within their genomes that are expressed during infection. The question that remains to be answered is whether *P. infestans* isolates employs gene silencing to restrict the expression of specific effectors. Sequencing of small RNAs could yield insights as to which effectors, if any, are affected in this way. Progress of this work would be presented.

520. *Phytophthora* phospholipase D genes and their role in plant cell degradation

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Plant pathogens secrete a large repertoire of proteins that may have a role in pathogenicity such as modulation of host defense (e.g. effectors) or degradation of host tissue. *Phytophthora infestans*, the potato late blight pathogen, possesses genes encoding secreted proteins with a phospholipase D (PLD) catalytic domain and this suggests that the *Phytophthora* secretome comprises this class of enzymes. PLD catalyzes the hydrolysis of membrane phospholipids leading to the production of phosphatidic acid and a free headgroup. Phosphatidic acid is a key player in the arena of cellular signalling. It is involved in many processes, including G-protein regulation, protein phosphorylation, transcription, cell proliferation and growth. PLD activity and phosphatidic acid production in plants is also correlated with membrane degradation during senescence and wounding. *Phytophthora infestans*, has 18 genes encoding PLDs. One PLD is a universal PLD that is present in all eukaryotes. A second one has homology to a novel class of PLD(-like) proteins. The other sixteen, divided over 4 subfamilies, are more diverged from known PLDs in eukaryotes and unique for *Phytophthora*. Two of these sub- families are small, secreted proteins. We detected PLD activity in exudates of *Phytophthora* and showed that these exudates are capable of degrading lipid vesicles. Transient *in planta* expression of the genes encoding secreted PLDs resulted in a calcium dependent induction of cell death responses. This could point to a function in host membrane modification and/or degradation. Further molecular and biochemical characterization of the various *Phytophthora* PLDs is in progress.

521. Brown colour development in the edible mushroom *Agaricus bisporus* infected with mushroom virus X

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Agaricus bisporus is prone to a viral disease caused by a complex of double- stranded RNAs known as mushroom virus X (MVX). MVX causes white mushrooms to produce a brown cap colour as they develop or during subsequent post-harvest storage.
This project focuses on how mushrooms undergo virus-infected browning. The approach was to screen for and identify genes involved in virus-induced browning, with a view to understanding how the virus-host interaction leads to browning symptom development. Gene expression differences between virus-infected brown and white mushrooms were characterised using Suppression Subtractive Hybridisation (SSH). 197 SSH 'virus- response' genes were incorporated into a custom *A. bisporus* micro-array to identify browning-related genes on the basis of differential expression in symptomatic mushrooms. 110 up- and down-regulated genes were identified as common to the majority of infected samples tested. Ten transcripts were found to be extremely highly up- regulated (148- to 4019-fold) in all virus-infected brown samples compared to non- infected controls. Quantitative reverse transcriptase-PCR analysis confirmed the micro- array results. Current work is underway to identify the function and origin of these highly up-regulated genes (*Agaricus* or MVX), and whether they are driving the browning reaction or a response to it.

522. *Candida albicans* cell surface superoxide dismutases degrade host- derived reactive oxygen species to escape innate immune surveillance

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The clinical spectrum *Candida* spp. ranges from mucocutaneous infections to systemic, life-threatening diseases in immunocompromised patients. One of the immediate early responses of host phagocytes challenged by fungal pathogens is the production of reactive oxygen species (ROS), which are important in inflammatory reactions but also aim at destroying invading pathogens. Using quantitative real-time ROS assays based on chemiluminescence, we show here that both yeast and filamentous forms of the opportunistic human fungal pathogen *C. albicans* trigger ROS production in primary innate immune cells such as macrophages and dendritic cells. Through a reverse genetic approach, we demonstrate that co-culture of macrophages or myeloid dendritic cells with *C. albicans* cells lacking the superoxide dismutase Sod5 leads to massive extracellular ROS accumulation in vitro. Notably, ROS accumulation was further increased in co-culture with fungal cells devoid of both Sod4 and Sod5. Survival experiments show that *C. albicans* mutants lacking Sod5 and Sod4 exhibit a severe loss of viability in the presence of macrophages in vitro. The reduced viability is not evident with macrophages from *gp91phox*$^{-/-}$ mice defective in the oxidative burst activity, demonstrating a ROS-dependent killing activity of macrophages targeting fungal pathogens. These data show a physiological role for cell surface SODs in counteracting the oxidative burst reaction, and suggest a mechanism whereby *C. albicans*, and perhaps many other microbial pathogens, can evade the host immune surveillance in vivo.

523. How oomycete and fungal effectors enter host cells.

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Oomycete and fungal plant pathogens produce effector proteins to condition host tissue for susceptibility. Many of these proteins can enter into the cytoplasm of plant cells, where they interfere with plant defense signaling and may also be recognized by intracellular plant resistance gene products. We have shown previously that conserved RXLR-dEER motifs in the N-terminus of several oomycete effectors enable these proteins to enter plant cells in the absence of any pathogen machinery. However, identification of motifs that fungal effector proteins to enter host plant cells have so far eluded identification. We carried out extensive mutagenesis of the oomycete RXLR-dEER motifs to improve our understanding of the spectrum of amino sequences that can enable host cell entry. Using this information, we have identified variant RXLR sequences in the N-termini of many fungal effectors, and have shown that they can enable host cell entry. Furthermore, we have identified a family of receptor molecules in plant cells that bind both oomycete and fungal N-terminal domains. Both the strength of binding and the specificity of binding are affected by mutations in the RXLR motif. This family of receptor molecules is also conserved in animal cells, and we have shown that the PEXEL motif of Plasmodium effectors ($RXLX^{E}/_{Q}$) can also bind to these receptors. Thus effectors from three different kingdoms of eukaryotic pathogens have evolved, convergently, the ability to target a highly conserved receptor family in eukaryotic hosts. The nature and location of the receptors support the hypothesis that the effectors enter host cells via receptor-mediated endocytosis.

524. *AVR-Pia* of *Magnaporthe oryzae* encodes a protein with a secretion signal sequence

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Studying the relationships between AVR and R genes is the main factor for understanding the mechanisms of mutation of host specificity. We therefore aimed to clone and analyze the *AVR-Pia* in *Magnaporthe oryzae* that exhibits avirulence toward the *Pia* resistance gene. We have already reported that *AVR- Pia* is located in the 1.2-kb DNA region (Vm), derived from 46F3, a positive cosmid clone insert found in Ina168 but not in Ina168m95-1 host specific mutant. Open reading frame analysis showed that the Vm region has several ORFs and the longest ORF is 255 bp in length. The ORF expressed by *TrpC* promoter, however, didn'ft complement avirulence. Then, Vq, a new 702-bp region that contains the 5'f end of the complimentary region Vm to the 3'f end of the ORF, was cloned. By virulent assays, this region also contains *AVR-Pia*. Frame shift mutations were then introduced into this region by inserting a total of six single bases into different locations of the ORFs. Only the two fragments with mutations in the 255-bp ORF showed virulence to Aichiasahi. It was finally concluded that *AVR-Pia* is the 255-bp ORF, which contains a predicted 19- amino acid signal peptide and was found to have a weak similarity with the bacterial cytochrome c family protein.

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525. *GzSNF1* is required for normal sexual and asexual development in the ascomycete *Gibberella zeae*

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The sucrose non-fermenting 1 (SNF1) protein kinase, a yeast homologue of mammalian AMP-activated protein kinase, plays a main role in transcriptional activation and repression of gene expression. Recent studies revealed that SNF1 genes are essential for disease development by pathogenic fungi such as *Sclerotinia sclerotiorum*, *Cochliobolus carbonum*, and *Fusarium oxysporum*. To elucidate the role(s) of *SNF1* in *Gibberella zeae*, we deleted its homologue (*GzSNF1*) using a gene replacement strategy. Mycelia growth of the delta- *GzSNF1* mutants was reduced by about 21~74% on diverse carbon sources. Infection assays on barley revealed that progression of necrosis in plants infected by the delta- *GzSNF1* mutants was considerably delayed compared with those by its wild-type progenitor. Especially, the delta- *GzSNF1* mutants showed a reduced expression of several genes encoding cell wall degrading enzymes such as XYL1, XYL2, and PL1. The most distinct phenotypic changes were in sexual and asexual development. The delta- *GzSNF1* strains, when selfed, produced fewer sexual fruiting bodies (perithecia) carrying abnormal sexual spore (ascospore). Also, delta- *GzSNF1* mutants produced fewer asexual spores (conidia), which contained shorter and curved on both sides with poorly defined septa. Furthermore, germination and nucleation of both ascospores and conidia were delayed in delta- *GzSNF1* mutants in comparison with those of the wild-type strain. To determine whether GzSNF1 localizes to nuclei, we made strains expressing GzSNF1- GFP fusion proteins and monitored the intracellular distribution of the GFP fluorescence in the strains by fluorescence microscopy. Base on the results of expression and localization of GzSNF1, we propose that GzSNF1 is critical for normal sexual and asexual development in addition to virulence and utilization of alternative carbon sources.

526. Small GTPases in *Botrytis cinerea*: impact on differentiation and pathogenicity

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The necrotrophic plant pathogen *Botrytis cinerea* is able to infect more than 200 host plants, including important crops and ornamentals.Using a broad candidate gene approach, many signaling components were identified and characterized in B. cinerea that are critical for successful plant infection. Since ROS play a major role in fungal differentiation and are known to be involved in plant-host interaction one focus of our current work is the relationship between known signaling pathways and ROS dependent mechanisms, to understand the complex regulatory network that triggers virulence and differentiation in B. cinerea.We presently characterize small GTPases of the Ras superfamily in B. cinerea: BcRAS1, BcRAS2, BcRAC and BcCDC42 and their influence on other signal transduction mechanisms. Recently, it was shown that the small GTPase BcRAS2 is linked to cAMP signaling in B. cinerea (1).BcRAS1 and BcRAC seem to act in the same signaling pathway as the phenotypes of the corresponding deletion strains is almost identical. RAC homologues are known to be involved in activating the NADPH oxidase complex; this complex was also identified in B. cinerea (2) and we are currently analyzing the influence of BcRAC on this ROS generating system.We also focus on the fungal oxidative stress response systems,including the MAP kinase BcSAK1 (3), and on cross-talks between the different cascades involved. 1. Schumacher, Kokkelink et al.MPMI(2008)21:1443-59 2. Segmüller et al. MPMI(2008)21:808-19 3. Segmüller et al. Euk.Cell(2007)6:211-21.

527. Secreted effectors of the tomato leaf mould fungus *Cladosporium fulvum* are virulence factors that target host defense

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Cladosporium fulvum is a biotrophic fungal pathogen that causes leaf mould of tomato. Inside the leaf, *C. fulvum* does not penetrate host cells or develop haustoria but remains confined to the intercellular space between mesophyll cells. Ten effector proteins that are secreted during host colonization have been identified so far. We have recently revealed virulence functions for a number of these effectors. It was previously shown that the Avr2 effector interacts with, and inhibits, the tomato cysteine protease Rcr3 which, in compliance with the guard hypothesis, is required for immunity mediated by the tomato resistance protein Cf-2. However, in compatible interactions Avr2 inhibits several additional extracellular host cysteine proteases that are required for host basal defense. Also the recently identified secreted effector Ecp6 is a true virulence factor, as RNAi- mediated gene silencing compromised fungal virulence on tomato. Intriguingly, all previously identified *C. fulvum* effectors are unique as no clear homologs have been identified in other organisms. Ecp6 is an exception to this rule, with clear homologs in many fungal species. Ecp6 contains LysM domains that have been implicated in carbohydrate binding and we speculate that Ecp6 plays a role to dampen host immune responses. A detailed characterization of the secreted effectors will be presented.

528. Analysis of secreted and cell wall-associated proteins of the dermatophyte *Arthroderma benhamiae*

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The zoophilic pathogen *A. benhamiae* was selected to serve as a model organism to elucidate general pathogenicity mechanisms of dermatophytes. During infection the fungus is confronted with the skin complement as the first immunological defense system. To obtain an overview about secreted proteins of *A. benhamiae*, submerse culture supernatants and LiCl cell wall extracts were analysed by 2D-PAGE and MS. Culture analysis revealed a strong induction of proteolytic activity by keratin as nutrient. Numerous secreted proteases, different hydrolases and functionally uncharacterised proteins were identified by proteome analysis. Variation of the secretome composition at different time points was observed. In addition, secreted proteases of *A. benhamiae* are involved in immune evasion by degrading components of complement. Thus, opsonization of the fungus is prevented and the fungus is protected against phagocytosis. Taken together, this analysis resulted in a comprehensive proteome map of the *A. benhamiae* secretome and gives insights into pathogenicity, time and spatial distribution of secreted proteins.

529. Role of reactive oxygen species and stress signaling pathways in development and virulence of *Cochliobolus heterostrophus*.
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Reactive oxygen species (ROS) induce cellular stress pathways, as well as provide developmental cues. Host and environmental signals modulate sexual and asexual sporulation, appressorium formation and virulence of the maize pathogen *Cochliobolus heterostrophus*. To study the signaling network linking stress and developmental responses, we identified genes encoding NADPH oxidases (NOX), superoxide dismutases (SOD), Rac GTPases and upstream members of the MAPK modules. Search of the *C. heterostrophus* strain C5 genome indicates 3 NOX, 3 Cu/Zn SOD and 3 Fe/Mn SOD genes. *noxA*, *noxB*, and *noxC* mutants grow normally; noxA mutants show reduced pigmentation and sporulation. Mutants in *NOXR*, encoding a regulatory subunit common to all NOX complexes, show, in addition to the phenotypes of *noxA*, drastically reduced virulence and less staining for superoxide when germinated on a glass surface. ROS production thus appears important for virulence. Mutants in *SOD1* and *SOD2*, predicted to encode Cu/Zn superoxide dismutases, show no obvious phenotypes, while preliminary evidence suggests the Fe/Mn type Sod3p is essential for normal development. We make some predictions for upstream kinases in the Hog1p cascade linking stress signals to gene expression. Stress response pathways may detect and respond to ROS, the level of which, in turn, depends on the balance between production and breakdown.

530. Recognition of *Phytophthora infestans* RXLR-dEER effectors by resistance proteins is triggered by C-terminal domains comprising W motifs
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The *Phytophthora infestans* avirulence genes *PiAvr1* and *PiAvr4* encode RXLR-dEER effector proteins and belong to a family of oomycete avirulence homologs (*Avh*). Avh proteins are rapidly evolving but nevertheless, the majority has recognizable C-terminal motifs (Jiang et al. 2008 PNAS). *PiAvr4* was isolated by positional cloning. Loss of avirulence on *R4* potato is caused by frame shift mutations resulting in truncated PiAvr4 proteins (van Poppel et al. 2008 MPMI). The genomic region harboring *PiAvr4* shows conserved synteny with *Phytophthora sojae* and *P. ramorum* but *PiAvr4* itself is located on a 100 kb indel that breaks the conserved synteny, and is surrounded by transposons. In the C-terminus PiAvr4 has three W motifs and one Y motif. W2 in combination with either W1 or W3 triggers necrosis in potato plants carrying resistance gene *R4*. *PiAvr1* was isolated by anchoring *Avr1*-associated markers on the genome sequence. This lead to a 800 kb region with seven *Avh* genes, one of which is *PiAvr1*, the counterpart of resistance gene *R1*. Also PiAvr1 has W and Y motifs. Domain swapping revealed which motifs determine avirulence on *R1* potato. Analysis of the role of PiAvr1 and PiAvr4 in virulence is in progress.

531. Killing of *Aspergillus fumigatus* conidia and germlings by phagocytic cells is dependent on initial cell to cell adherence.
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Aspergillus fumigatus (Afu) is a fungal pathogen that causes invasive asperigillosis in immunocompromised patients. When immunocompetent individuals aspirate Afu conidia, conidia fail to germinate and are rapidly killed by alveolar macrophages. Killing is dependent on reactive oxygen species (ROS) and lysozomal enzymes. However, the essential role of ROS in killing of conidia is confounded by findings that Afu conidia are killed by neutrophils defective in the production of ROS. To understand the mechanisms of virulence used by Afu, we characterized the adherence, phagocytosis and killing of Afu conidia in human monocytes and mouse alveolar macrophages. Phagocytosis of conidia was observed as early as 5 min, and increased linearly with time. Conidia were more effectively phagocytosed at higher multiplicities of infectivity (MOI) to a maximum of 22% at MOI 9:1. Increasing the MOI further had a negative effect on phagocytosis. Opsonization of conidia with mouse serum increased the amount of phagocytosis more than two-fold, suggesting that this is an important defense mechanism against Afu. Our results show that host cells kill conidia as early as 5 min, and greater than 60% of conidia are killed after 1 h. Previous studies showed that greater than 85% of adhered conidia are phagocytosed by host cells. Our results suggest that Afu might be able to evade host defenses by preventing initial attachment onto phagocytic cells, thereby evading the respiratory burst and other antimicrobial processes.

532. Reactive oxygen species- *Botrytis cinerea*'s friends or foes during host infection?
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Botrytis cinerea is a phytopathogenic ascomycete infecting a broad range of dicotyledonous plants. In the course of infection the necrotrophic fungus is exposed to reactive oxygen species (ROS) released by its host in the "oxidative burst", an early plant defence reaction. But *B. cinerea* even produces ROS itself *in planta*. This raises the question how the pathogen senses and responds to the host defence reaction. Do ROS released by the plant harm the pathogen at all? Investigations on the AP-1 transcription factor Bap1 revealed its role as a pivotal regulator of ROS detoxification in axenic culture under exposure to H_2O_2 and menadione, an intracellular ROS generator. Macroarray analysis revealed 99 H_2O_2-induced Bap1 target genes. Besides other gene products, it controls transcription of several ROS degrading enzymes as well as of the thioredoxin and the glutaredoxin system, regulators of the cellular redox status. Interestingly, Bap1 is not essential for pathogenesis and its target genes are not expressed on the host 2 days post infection indicating a minor role for H_2O_2 degradation during infection. However, other redox- regulators like the SAPK BcSak1 or the Nox complex are essential for normal virulence. Therefore, we focus on the characterisation of factors involved in ROS signalling in order to connect different pathways and to elucidate their regulation.

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533. A transcriptional analysis of rice leaves infected by *Magnaporthe grisea* provides new insights on the role of the fungal secretome and of chromatin remodelling in pathogenicity

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A transcriptional analysis of rice leaves infected by*M.grisea* (5 days after inoculation) to identify 1851 genes expressed during infection, 244 being specific. These infection specific genes mainly encode secreted proteins (40%) as well as a small group of chromatin remodelling proteins. To test that chromatin remodelling is important for infection, we deleted the four *M.grisea*. histone deacetylases. ,Two mutants are reduced in pathogenicity while another fully non pathogenic. Since *M.grisea* secretes a large number of proteins during infection, we developed a proteomic analysis of its secretome. Using 2D and 1D gel electrophoresis, we identified 221 proteins from in vitro culture filtrates including 135 proteins with signal peptides. 40% of these proteins are expressed during infection. Among the 38 small secreted proteins identified, 15 are expressed during infection (38%) and are candidate effectors. They will be studied in the in vitro secretome for their effect on rice (IRMA project) and their role in infection.

534. Interaction of *Aspergillus fumigatus* with the human complement system

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The complement system plays a crucial role in the defense against pathogenic microorganisms. This study describes two mechanisms of complement evasion of the opportunistic human-pathogenic fungus *Aspergillus fumigatus*. The binding of complement regulators to different developmental stages of *A. fumigatus*, as well as to a pigmentless *pksP* mutant, was analyzed and distinct binding patterns were observed. Conidia showed strong binding of three regulators of complement activation: factor H, FHL-1, and plasminogen. In contrast to conidia, hyphae showed only weak binding of complement regulators. The pigmentless *pksP* mutant, which produces white conidia and which proved to be attenuated in virulence in a mouse infection model, was found to bind no factor H and plasminogen. Remarkably, hyphae inactivate complement in a factor H independent way. Culture supernatant of *A. fumigatus* showed complement cleavage activity. Key components of the complement activation cascade (C3, C5 and C4) were efficiently degraded by a secreted protease. These two independent mechanisms might help *A. fumigatus* to evade the attack of the human complement system.

535. The biotrophic interfacial complex and the secretion of effector proteins into host cells during rice blast disease.

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The hemibiotrophic fungus *Magnaporthe oryzae*, causing the globally important rice blast disease, grows inside living rice cells by producing specialized invasive hyphae (IH) that are encased in a plant-derived extra-invasive-hyphal membrane (EIHM). Little is known how the fungus secretes effector proteins and delivers them into host cytoplasm across the EIHM to control host cells for successful disease, or to trigger host resistance. Using live-cell imaging and correlative light and electron microscopy, we identified a novel structure, the Biotrophic Interfacial Complex (BIC) that is suggested to mediate effector secretion. Each time the fungus enters a living rice cell, the BIC forms at the tip of the filamentous hypha and then is repositioned to the side of the first IH cell that differentiates from this filamentous hypha. BICs are a highly localized structure that consists of a complex aggregation of membranes and vesicles. Several blast effector proteins including AVR-Pita1, PWL1, and PWL2 are secreted into BICs when expressed by the fungus as fusion proteins with different fluorescent proteins (EYFP, EGFP, or mRFP). AVR-Pita and PWL signal peptides alone are able to direct this localized secretion. This process involves a classical ER-secretory pathway, because addition of the tetrapeptide ER retrieval signal to the C-terminus of AVR-Pita signal peptide:EGFP resulted in ER retention of fusion protein. EGFP expressed with AVR-Pita promoter and secreted with a non-effector signal peptide (from cutinase) did not show preferential BIC localization, suggesting that effector protein signal peptides contain information that controls BIC localization. We hypothesize that effector proteins are secreted first into BICs and then into the host cytoplasm.

536. Adaptation to pH and role of PacC in the rice blast fungus *Magnaporthe grisea*

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Perception of external environment is important for successful interactions between plants and fungi. In fungi, a specific and conserved pathway is responsible for pH signalling. In the rice blast fungus *Magnaporthe grisea*, the role of this pathway in cell physiology and development of the fungus in planta is still unknown. Seven proteins, including the putative transmembrane sensor protein PalH and the transcription factor PacC, are part of the signal transduction machinery for pH sensing. Alkaline pH leads to PacC activation and its transfer into the nucleus where it activates transcription of a specific set of genes expressed under alkaline conditions. Here we report on the influence of ambient pH on *M. grisea*'s biology. The role of PacC was investigated through deletion of the corresponding gene. Several phenotypes were studied in the mutant strain, including growth rate, penetration and ability to infect host plants. This enabled the investigation of the role of pH sensing in *M. grisea*'s development cycle. Furthermore, a gene expression profiling analysis of the *pacC* mutant was initiated and revealed the involvment of PacC in both "acidic" and "alkaline" genes expression. Taken all together, the results indicate that this pathway is important for the fungus adaptation to an alkaline environment and that it plays a role in the fungus pathogenicity when tested on barley plants.

537. RBP35, a *Magnaporthe oryzae* RNA binding protein involved in pathogenicity, affects melanin biosynthesis.

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Magnaporthe oryzae is able to infect leaves and roots of cereal plants. To elucidate the different molecular mechanisms involved in the M. oryzae root infection process, we screened a *M. oryzae* T-DNA insertional library to identify mutants impaired in root colonisation. The mutant M35 was considerably affected in its ability to cause disease on roots and it also showed reduced virulence on leaves. M35 has undergone insertional inactivation of the *RBP35* gene, encoding a predicted RNA binding protein containing a RNA recognition domain (RRM) and several RGG motifs. The M35 phenotype has been confirmed by targeted gene disruption of *RBP35* gene in the wild-type strain. The full length cDNA has been characterised by a combination of 5' and 3' RACE and sequencing strategies. Two polyA sites were identified by 3' RACE and the two corresponding transcripts have been detected by Northern analysis. Their abundance does not significantly vary under different growing conditions. On the contrary, antibodies against RBP35 recognise two proteins that seem to behave differently in various growing conditions. The smaller isoform is the most abundant. Proteomics comparison of the M35 mutant and the corresponding WT strain Guy11 have shown that RBP35 affects secondary metabolite pathways, in particular melanin and flavonoid biosynthesis. Further metabolomics investigations are in progress to better characterise this regulation. RBP35 is likely to be a post-transcriptional regulator implicated in fungal development and pathogenesis and seems to be post-transcriptionally regulated.

538. Lysine biosynthesis is involved in *Fusarium graminearum* virulence on wheat

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F. graminearum is a well known fungal pathogen of maize, wheat and other small grain cereals. The amino acid lysine is synthesized via the alpha-aminoadipate pathway in lower eukaryotes. A key reaction is the formation of alpha-aminoadipate semialdehyde, mediated by alpha-aminoadipate reductase (EC 1.2.1.31). In yeast, two distinct genes, *LYS2* and *LYS5* are required for this reaction. The *F. graminearum FgLYS2* ortholog was identified by searching the MIPS *Fusarium* Database. The gene model of *FgLYS2* was verified by cloning the corresponding *FgLYS2* cDNA. To verify the proposed function, *FgLYS2* was used to complement a *lys2* deficient *S. cerevisae* strain. To study the relevance of lysine-biosynthesis in pathogenicity, a *FgLYS2* deletion strain was constructed. The virulence of a *F. graminearum* wildtype strain and a *lys2* strain were visually assessed on flowering wheat heads by counting the bleached and wilted spikelets. After infection with the wildtype, the whole wheat head showed severe symptoms. In plants infected with the *lys2* strain, symptoms were restricted to the inoculated spikelet. This result shows that lysine biosynthesis is essential for spread and full virulence on the plant, and is therefore a potential target for *Fusarium* control.

539. Homologues of the *Cladosporium fulvum* effector proteins are present in Mycosphaerella species

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Cladosporium fulvum is a non-obligate biotrophic fungal pathogen that causes leaf mould of tomato. Until now, ten effector proteins have been identified from *C. fulvum* that historically have been divided into avirulence (Avrs: Avr2, Avr4, Avr4E and Avr9) and extracellular proteins (Ecps: Ecp1, Ecp2, Ecp4, Ecp5, Ecp6 and Ecp7), and whose recognition in tomato is mediated by their cognate Cf (for *C. fulvum*) resistance proteins. Although demonstrated for only some of them, all Avrs and Ecps are assumed to be virulence factors. Avr2 is an inhibitor of apoplastic plant cysteine proteases, while Avr4 encodes a chitin-binding protein that protects chitin present in the cell walls of the fungus against the deleterious effects of plant chitinanes during infection. Ecp6 contains carbohydrate/chitin-binding LysM domains that putatively bind chitin fragments released from fungal cell walls during infection and prevents them from inducing basal defense responses during infection. Despite the fact that the interaction between *C. fulvum* and tomato is a model to study gene-for-gene based interactions, so far no homologues of the *C. fulvum* effector proteins have been identified in other fungal species. However, by mining of sequenced fungal genomes for homologous nucleotide sequences and protein motifs, we have now identified for the first time homologues of the *C. fulvum* Avr4, Ecp2 and Ecp6 effectors in other fungal pathogens, including *Mycosphaerella fijiensis*, *Mycosphaerella graminicola* and *Cercospora nicotianae*. Recently we have tested whether the three *M. fijiensis* homologues are truly functional homologues of the three *C. fulvum* effectors. We will report on the exciting results that have been obtained from these studies.

540. Identification and characterization of *Fusarium verticillioides* Fsr1-interacting proteins associated with maize stalk rot pathogenesis

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Fusarium verticillioides is one of the key pathogens causing stalk rot in maize. However our understanding of stalk rot pathogenesis is still limited. Previously, we identified the *F. verticillioides FSR1* gene and showed it plays an important role in fungal virulence and female fertility. The predicted Fsr1 protein contains multiple protein binding domains - a caveolin binding domain, a coiled-coil structure, a calmodulin binding motif in the N- terminus and a WD40 repeat domain in the C-terminus. We also determined that the coiled-coil structure is essential for stalk rot virulence whereas the WD40 repeat is dispensable. Coiled-coil structure is known to mediate protein-protein interactions *in vivo*, and our premise is that this interaction triggers downstream gene signaling associated with stalk rot virulence. In this study, our aim was to identify and characterize putative Fsr1 coiled-coil structure-binding proteins using yeast-two hybrid screening approach. Two bait constructs, a Fsr1 cDNA encoding the N-terminus region and a Fsr1 cDNA encoding the N-terminus region without the coiled-coil motif, were used as baits to screen a *F. verticillioides* cDNA library. We are in the process of performing *in silico* subtraction and further *in vivo* and *in vitro* verification of yeast-two hybrid screening results. Furthermore, detailed functional characterization of the genes identified in this screen is in progress.

541. Genome wide analysis of the secretome repertoire in the poplar rust pathogen *Melampsora larici-populina*

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The foliar rust caused by *Melampsora larici-populina* is the main disease affecting poplar plantation in northern Europe with severe economic losses. Epidemiology of the disease and plant defense response have been studied but very little is known about fungal molecular mechanisms during the infection process. In the wake of the *Populus* genome sequencing, the ~100 Mb genome of *M. larici-populina* have been sequenced (7X depth). The analysis of this genome is a great opportunity to identify loci coding for the arsenal developed by the rust fungus to penetrate and exploit its host. It has emerged from different studies that secreted proteins are expressed by biotrophic fungi during the infection process and particularly during formation of the haustorial structure. Such secreted proteins may interact with or manipulate the host plant during the infection. In the frame of the annotation of the poplar rust genome sequence, we identified homologs of previously described haustorially expressed genes including avirulence factors from other rust species and many *M. larici-populina* specific genes. Expression analyses have been caried out during time-course infection of poplar leaves using NimbleGen *Melampsora* oligoarrays and RT-qPCR. Several secreted proteins encoding genes are specifically expressed during host-infection representing a potential reservoir for new rust effectors.

The Melampsora larici-populina genome sequence was produced by the US Department of Energy Joint Genome Institute

542. Vectors to study localization of Epichloe festucae gene products in the symbiotum

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In the past 10 years, the autofluorescent proteins (AFPs) have been extensively developed and applied in prokaryotic and eukaryotic organisms. In the filamentous fungi, AFPs have been applied to study life cycle, protein secretion, signal transduction, metabolic pathways, fungus-host interactions, etc. For Epichlo?festucae, a model species for symbioses of endophytes with C3 grasses, GFP expression has revealed intercalary hyphal extension in elongating grass leaves, and different growth patterns of wild-type and mutant strains in symbio. The plasmid, pCT74, generously provided by Dr. L.M. Ciuffetti, includes a GFP coding sequence controlled by the Pyrenophora tritici- repentis ToxA gene promoter. E. festucae transformed with pCT74 was visualized in host leaves and meristematic tissues. Our objective is to use dual AFP labeling, whereby GFP is used to track hyphal growth, and differently colored AFPs are used to accurately assess in symbio expression and localization of E. festucae gene products of interest. To this end, the selectable marker in pCT74 was replaced with a loxP-flanked hygromycin- resistance (hph) gene cassette so that it can be eliminated easily from transformed E. festucae (see poster by Florea et al.). Then, the same marker can be used for repeated transformation with genes of interest fused with other AFP coding sequences. To facilitate construction of such fusion constructs, a bacteriophage att site homologous recombination system (GATEWAY) was employed for fusion of target genes, including their native promoters, with AFP coding sequences. This set of expression vectors will be very useful for analysis of E. festucae genes and gene products on a genome-wide scale.

543. Comparative Gene Expression Analysis of *Fusarium graminearum* in *Triticum aestivum* and *Oryza sativa* spp. japonica

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Negligible amounts of Type B trichothecene mycotoxins accumulate in *Oryza sativa* spp. japonica infected with *Fusarium graminearum* relative to *Triticum aestivum* inoculated with an identical strain of the fungus. In *T. aestivum*, expression levels of all genes increased between each of three time points, while expression levels of genes in *O. sativa* remained relatively constant - results well- correlated with symptom progression observed on both plants. To identify differential fungal gene expression patterns that could lead to differences in toxin accumulation and symptom onset and development, analyses of gene expression were conducted during infection of each plant using *F. graminearum* Affymetrix GeneChips. Expression profiles were generated for time points 48, 96 and 192 hours after inoculation of plants and analyses revealed subsets of genes expressed only in *T. aestivum* (232) and *O. sativa* (21). Classification of these genes using MIPs FunCat categories revealed that 109 genes expressed exclusively in *T. aestivum* fell into the Unclassified Protein category; 13 genes expressed exclusively in *O. sativa* were similarly classified. Non-coding upstream regions of genes expressed exclusively in *T. aestivum* or in *O. sativa* revealed an enrichment of nucleotide sequences: acgtca and ccccgc in *T. aestivum* and aaccac and acaacg in *O. sativa*, suggesting potential regulatory significance.

544. Studies on the *RETRO5, RETRO6*, and *RETRO7* retrotransposons of the rice blast fungus.

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During the infection process of the rice blast fungus, it comes in contact with different stresses from the host plant as a result of hypersensitive reaction. The fungus abundantly harbors a variety of transposable elements that are potential causes for its high genetic variability. The lack of available data on the characteristics of *RETRO5, RETRO6*, and *RETRO7* retrotransposons of the rice blast fungus prompted our investigation on the expression of these elements as an effect of using methyl viologen (MV), methyl methane-sulfonate (MMS), and heat shock (HS) as stress treatments through Northern and real time-PCR analysis. Southern analysis was also done to determine the transpositional activities of the elements as affected by the stress treatments. Results showed that induced expressions of *RETRO6* and *RETRO7* with 10.0mM MV treatments were not directly related to insertion of new copies of both elements in the genome of *Magnaporthe oryzae* Ina168 single- conidium isolates. *RETRO5* expression, on the other hand, was induced as well as its retrotranspositional activities enhanced with 0.15% MMS treatment. With these interesting characteristics of *RETRO5*, more characterization experiments of the element were done, such as cloning and determining its whole sequence and the flanking region sequence of its full length insert on Ina168, to gain more knowledge on the element.

545. New tools for the advancement of *Mycosphaerella graminicola* research.
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Mycosphaerella graminicola is a major pathogen of wheat world wide, causing the disease Septoria leaf blotch. Infection results in severe yield losses, especially when the disease is not adequately controlled. Generating a greater understanding of the biology, infection process and pathogenicity of *M. graminicola* is fundamental to increasing our knowledge of this pathogen.
The development of genomics tools for *M. graminicola* over recent years have enabled significant advances to be made in this field to date.
Here we report further developments to these tools with: i)The first report of the successful use of a second selectable marker, G418, for *M. graminicola* transformation ii)The generation of a strain of *M. graminicola* in which the *MgKu70* gene has been deleted. iii)The ability to efficiently target and generate precise manipulations or mutations of genes in the genomic context in *M. graminicola*
The *Ku70* gene is involved in the non-homologous end joining of pieces of DNA; in other filamentous fungi it has been reported that when it is disrupted, rates of homologous recombination increase to >90%. Results will be discussed showing this is also true for *M. graminicola*.
The ability to efficiently perform targeted manipulations of genes in the genomic context, such as insertion of point mutations or precise insertions or deletions, now allows hypotheses to be tested on the importance of key residues in the function of a gene. This will be exemplified with data showing the effect point mutations may have on the sensitivity of *M. gramincola* to fungicides. Results from these experiments also highlighted an interesting observation about the nature of homologous recombination in *M. graminicola* which will be discussed.

546. Ferroxidases in *Cryptococcus neoformans*: their roles inside macrophages and in antifungal susceptibility.
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Iron acquisition is a critical aspect of the virulence of many pathogenic microbes during growth within a mammalian host, and iron limitation is considered a primary defense mechanism by host cells. We study iron acquisition systems and iron-related regulatory mechanisms in the human fungal pathogen *Cryptococcus neoformans*. Our recent major discovery was the central role of the iron regulatory protein Cir1 in virulence, and we have also characterized a siderophore transporter Sit1 as well as the iron permeases Cft1 and Cft2. We recently identified the *Cryptococcus* ferrioxidases Cfo1 and Cfo2 that are coupled with Cft1 and Cft2, respectively, in the reductive iron uptake system. Here we present data that identifies specific roles for Cfo1 and Cfo2. Cfo1 is required for the reductive iron uptake system that is responsible for utilization of transferrin, a known primary iron source for *C. neoformans* within a host. However, Cfo1 did not play any role in heme or siderophore utilization. We constructed GFP fusion proteins and found that expression of Cfo1 is induced by iron limitation and is mainly localized at the plasma membrane. Furthermore, we found that Cfo1 is preferentially expressed within murine macrophages and that the catalytic subunit of cAMP-dependent protein kinase (Pka1) influences its localization *in vivo*. Strains lacking *CFO1* displayed increased sensitivity to antifungal drugs fluconazole and amphotericin B. Interestingly, wild type sensitivity was restored by addition of exogenous heme. These results suggested that increased sensitivity is due to the reduced levels of intracellular heme, which is required by proteins of the ergosterol synthesis pathway. Finally, the *cfo1* mutant was attenuated for virulence in a mouse model of infection thus revealing the significance of Cfo1 in pathogenesis.

547. The role of mating type in virulence of the fungal pathogen *Cryptococcus neoformans*.
Laura H. Okagaki and Kirsten Nielsen

Cryptococcus neoformans is an opportunistic human fungal pathogen that afflicts immunocompromised individuals such as advanced HIV/AIDS patients. This encapsulated fungus is commonly found in the environment, however, when inhaled can cause acute lung infection and dissemination to the brain causing meningitis. There are two varieties of *Cryptococcus neoformans*: var. *grubii* and var. *neoformans*. Of these two serotypes, var. *grubii* is far more virulent, accounting for as many as 95% of cryptococcal infections worldwide. There are two mating types in *Cryptococcus*: **a** and alpha. When mice are experimentally infected intranasally with either var. *grubii* **a** or alpha alone, they exhibit similar disease progression. However, in mice infected with both **a** and alpha mating types simultaneously, a higher proportion of alpha cells are able to disseminate to the brain while equivalent numbers of **a** and alpha cells are found in peripheral tissues. Examination of lung tissue reveals a subset of cells that are 5 to 10 fold larger than the typical cell size. This observed cell enlargement is enhanced in coinfections - suggesting pheromone signaling may be involved in generation of large cells. Differential staining revealed a significantly higher proportion of enlarged mating type **a** cells during coinfection. These large cells have reduced phagocytosis by host immune cells, a necessary step in dissemination to the brain. Therefore, we hypothesize that pheromone signaling enhances mating type **a** cell enlargement and leads to decreased dissemination to the brain and ultimately reduced virulence.

548. *RIG1*: A gene homologous to the *Histoplasma capsulatum RYP1* regulator of virulence encodes a gene essential for pathogenicity in *Magnaporthe oryzae*. Amritha S. Wickramage, M. Alejandra Mandel and Marc J. Orbach, Department of Plant Sciences, Division of Plant Pathology and Microbiology, University of Arizona, Tucson, AZ 85721

In *Histoplasma capsulatum*, the gene *RYP1* encodes a transcription factor essential for yeast-form growth at 37°C and thus is essential for virulence. We identified a gene with homology to *RYP1* in the plant pathogen *Magnaporthe oryzae*, causal agent of rice blast disease. Gene replacement mutants of this gene were generated in *M. oryzae* strain 70-15. Deletion of this gene had no effect on the growth rate, colony morphology, sporulation, or appressorium formation of the mutant strains when compared to the parental strain. However, the mutants were non-pathogenic on susceptible rice cultivars Sariceltik and Maratelli. To test whether the pathogenicity defect was due to failure to penetrate host plants or failure to grow invasively, plants were infected following leaf abrasion and by direct spore injection. The mutant failed to cause disease in either case. We have designated the gene *RIG1*, for Required for Infectious Growth. These results indicate that *RIG1* encodes a pathogenicity factor that potentially regulates post-penetration growth in *M. oryzae*. The effects of *RIG1* deletion on appressorium penetration will be presented to determine whether both penetration and post-penetration growth is affected as occurs in *M. oryzae mst12* mutants. Expression analysis of the *rig1* mutant will also be presented as well as a spatial and temporal analysis of the expression of *RIG1*.

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549. Identification of *Fusarium virguliforme* induced genes by cDNA-AFLP during the interaction with glyceollin

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Fusarium virguliforme, previously known as *Fusarium solani* f. sp. *glycines*, is a soil-borne pathogen that causes Sudden Death Syndrome (SDS), one of the most destructive diseases of soybean in the United States. Little is known about the mechanisms governing the interaction between *F. virgulifomre* and soybean that result in SDS development. There is evidence that the ability of infected soybean plants to produce and localize the phytoalexin glyceollin is an important determinant of resistance of soybean plants to *F. virgulifomre*. The fungicidal effect of glyceollin on *F. virgulifomre* macroconidia germination and mycelia elongation was evaluated by exposing the fungus macroconidia and mycelia to different concentrations of glyceollin (150, 300, 600 µM). A cDNA-AFLP approach was used to identify genes that are differentially expressed in *F. virgulifomre* in the presence of glyceollin. Eight PCR- primer combinations were used to identify the differentially expressed transcript-derived fragments (TDFs) in the presence of the phytoalexin. The expression patterns of the corresponding genes were confirmed by quantitative Real time PCR (RT-PCR). Out of 16 identified TDFs, nine of them showed more than two fold change in expression levels in presence of glyceollin. This is a first report of *F. virgulifomre* genes expressed in the presence of glyceollin.

550. Isolation of new components of the *Epichloë festucae* NADPH oxidase complex

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The endophytic fungus *Epichloë festucae* systemically colonizes the intercellular spaces of perennial ryegrass to establish a mutualistic symbiotic association. We have recently shown that reactive oxygen species (ROS) produced by a specific NADPH oxidase isoform, NoxA, and associated regulators, NoxR and RacA, have a critical role in regulating hyphal growth in the host plant. Perennial ryegrass plants containing these mutants become severely stunted as a result of hyphal hyper-branching. In addition to the central Nox activation domain, NoxR has two protein interaction domains, an N-terminal TPR motif domain for the interaction with RacA and a C-terminal PB1 domain, indicating that NoxR acts as a scaffold for the Nox enzyme complex. Given PB1 domains are known to interact with other PB1 domains, fungal genome databases were screened for proteins containing this motif to identify potential candidates that interact with NoxR. Proteins identified included NoxR itself, Cdc24, Bem1 and a protein of unknown function. Yeast two-hybrid analysis showed that NoxR interacts with itself and with Cdc24, and Cdc24 interacts with Bem1. Site directed mutagenesis of conserved residues in the K and OPC motifs of the PB1 domains of NoxR, Bem1 and Cdc24, confirmed the specificity of these interactions. Infection of perennial ryegrass with the *f¢bem1* mutant caused mild stunting of the host plant and hyphal biomass increased. GFP fusions of NoxR and Bem1 preferentially localized to the hyphal tip and tips of emerging branches, providing further evidence that the Nox complex regulates branching and polarized tip growth of *E. festucae* in perennial ryegrass.

551. *Stagonospora nodorum* utilizes multiple host-selective toxins which act as effectors of pathogenicity to induce disease on wheat

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The *Stagonospora nodorum*– wheat interaction has been shown to involve multiple proteinaceous host-selective toxins (HSTs) that interact either directly or indirectly with dominant sensitivity gene products in wheat. Currently we have identified and published four HST–host gene interactions including SnToxA-*Tsn1*, SnTox1- *Snn1*, SnTox2-*Snn2*, and SnTox3-*Snn3*. In each case, toxin sensitivity is controlled by a single dominant gene. Using QTL analysis, significance of these interactions ranged from 18 to 63% of the disease variation, highlighting the importance of these interactions. Recently, two new HST-wheat sensitivity gene interactions have been characterized. A proteinaceous HST temporarily named SnTox4B was shown to be between 10 and 30 kDa and interact with the wheat gene *Snn1A* found on chromosome 1A. The SnTox1A-*Snn1A* interaction accounts for as much as 44% of the disease variation. Another proteinaceous HST temporarily named SnTox4B, also in the range of 10-30kDa interacts with a gene product identified on wheat chromosome 4B temporarily named *Snn4B*. The SnTox4B-*Snn4B* interaction has been identified in both hexaploid (bread wheat) and tetraploid (durum wheat) populations and has been shown to account for as much as 9% and 61% of the disease variability, respectively. To date we have accumulated solid evidence for six HST-host gene interactions as well as preliminary data for the presence of several additional significant interactions. This work establishes S. nodorum blotch as a model inverse gene-for-gene system where proteinaceous HSTs, acting as effectors of pathogenicity, interact with dominant host gene products resulting in a compatible disease interaction.

552. *Fusarium oxysporum* produced volatiles promote growth in *Arabidopsis thaliana*

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Fusarium oxysporum is a well-known soilborne plant pathogen notorious for causing wilt symptoms in a large number of plant species. However, not all isolates are plant pathogenic, and some of the nonpathogenic isolates even function as a biocontrol agent. We found, for the first time, that certain *F. oxysporum* strains produce volatile compounds that promote plant growth using the model plant *Arabidopsis thaliana*. We have identified a number of plant growth promoting isolates of *F. oxysporum* and trapped the volatiles produced by them through the use of SuperQ absorbent traps and are currently developing a procedure for studying and analyzing their effects on plant growth. A number of *A. thaliana* mutants defective in the hormonal regulatory pathways controlling plant growth are being employed in this analysis. The effect of these compounds on disease defense is being studied. We are also studying the chemical properties of those compounds through chemical analysis methods (Gas chromatography and Mass Spectrometry). The study of these volatiles will help us elucidate and comprehend plant-microbe interactions mediated through volatile compounds, especially the mode of action by which pathogenic fungi influence their hosts.

553. Urea amidolyase enhances virulence in a mouse model of disseminated candidiasis

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C. albicans produces intracellular urea amidolyase (Dur1,2) rather than urease for nitrogen utilization from urea. In order to investigate the role of urea amidolyase in candidemia, we disrupted the urea amidolyase gene *(DUR1,2)* in wild- type strain A72 and fully reconstituted it. Deletion of *DUR1,2* prevented growth when urea was the sole nitrogen source. The effect of deleting *DUR1,2* on the pathogenesis of disseminated candidiasis was examined using a mouse model. Deletion of *DUR1,2* significantly enhanced survival of infected mice compared with the wild type and reconstituted strains. The mechanism for reduced virulence of the *DUR1,2*mutant was examined by analyzing serum cytokine levels over time compared with the wild type strain. Infection by the *DUR1,2* mutant caused significant down regulation of IL-4 and IL-10 compared with the wild type at the sub-acute phase of the infection. This difference would enable more induction of protective the Th-1 subset of cytokines required for the resistance against fungal infection in mice infected with the mutant strain. This may also account for the more regulated pattern of TNF-alpha expression in mice infected with the mutant versus wild type strains, which would enhance protection against fungal infection. In addition, infection by the wild type strain caused significantly elevated G-CSF from 12 hours to 6 days post inoculation, whereas mice infected with the mutant strain had significantly elevated G-CSF levels only up to three days post inoculation. Therefore we conclude that, in addition to utilization of urea as a nitrogen source, Dur1,2 plays a pivotal role in altering host immune regulations to establish candidemia.

554. Developing a framework for understanding the interactions between a forest pathogen, *Grosmannia clavigera*, its mountain pine beetle vector and lodgepole pine host using the Illumina sequencing platform.

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Grosmannia clavigera, is a lodgepole pine pathogen and staining fungus that is specifically associated with the bark beetle, *Dendroctonus ponderosae* (mountain pine beetle, MPB). The MPB and its fungal associate are causing large-scale damage to conifer forests in northwestern North America. We have assembled a draft genome sequence for a reference isolate, and are characterizing genomic variation within the pathogen population by re-sequencing additional isolates. For the reference strain, we have used paired-end transcriptome sequencing (RNA-seq) to generate expression profiles for the fungus grown on artificial media, with and without phenolics or terpenes. With these data, we are annotating the draft genome sequence of the reference isolate and are identifying genes and gene families involved in the fungal pathogen's response to the tree's chemical defences.

555. Deletion of candidate genes encoding secreted proteins of *Colletotrichum graminicola* identifies a laccase as a virulence factor

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The hemibiotroph *Colletotrichum graminicola* is the causal agent of stem rot and leaf anthracnose on its host *Zea mays*. After penetrating host epidermal cells the fungus initially shows a biotrophic growth style that is followed by necrotrophy. During both phases, secreted proteins are assumed to play important roles to establish and maintain the interaction. We have used the Yeast Secretion Signal Trap (YSST) to identify more than 100 secreted proteins of *C. graminicola*. Only half of those exhibited similarity to proteins that have a known or a predicted function. The transcript levels were assessed by macroarray hybridization and qRT-PCR which indicated that many of the identified genes are induced by currently unknown host-derived compounds. To address the importance of genes identified by YSST for pathogenesis, we have chosen a dozen for deletion experiments. None of these genes seemed to be absolutely required for pathogenesis. However, when transformants carrying a deletion of a laccase gene were inoculated on whole corn plants or excised leaf segments, clearly reduced symptom development was observed. Currently, we are assessing the effects of deletion of a second laccase gene that was also identified by YSST.

556. *Trichophyton rubrum* gene expression profile during the process of keratin degradation

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Trichophyton rubrum is a cosmopolitan and anthropophilic fungus able to invade keratinized tissues, causing infection of human skin and nails. Once established in its host, it must scavenge nutrients for growth and development. The secretion of enzymes, such as keratinases, is a determining factor in the invasion and utilization of host tissues as source of nutrients, and it is often regulated by the ambient (extracellular) pH. Therefore, the mechanisms for installation and maintenance of host infection are probably dependent directly or indirectly on the environmental pH sensing. Furthermore, during *T. rubrum* growth on keratin the pH of the medium changes, reaching values higher than 8.0. This work aims to reveal *T. rubrum* differentially expressed genes during the process of keratin degradation, mimicking the conditions of infection, using cDNAs microarrays methodology. Therefore, cDNA clones were selected and spotted in membranes, using T. rubrum ESTs database generated by our laboratory. Bioinformatics softwares such as SAM and Cluster & tree-view were used, respectively, to identify genes with statistical differential expression and to make the dendograms. These studies contribute to the understanding of adaptive responses to environmental pH in *T. rubrum*. Furthermore it may also reveal genes involved in the pathogenic mechanisms of this dermatophyte, which will represent potential targets for the development of new antifungal drugs.

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557. The Set3 complex regulates penetration and infectious growth in *Magnaporthe grisea*

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The Set3 complex consisting of Snt1, Sif2, Cpr1, Hos2, Hos4, Hst1, and Set3 regulates early and middle sporulation genes in *Saccharomyces cerevisiae*. Our previous studies have shown that the *SIF2* homolog named *TBL1* is essential for plant infection in both *Fusarium graminearum* and *Magnaporthe grisea*. Using the affinity purification approach, we have identified several Tbl1-interacting proteins in *M. grisea*, including putative homologs of Snt1, Hos2, Hos4, Hst1, and Cpr1. The Set3 homolog exists in *M. grisea* and weakly interacts with Tbl1 in yeast two-hybrid assays. We have generated targeted deletion mutants of these genes in *M. grisea*. The *mgset3*, *mgsnt1*, and *mghos2* deletion mutants in *M. grisea* had similar phenotype with the *tbl1* mutant. These mutants were defective in plant infection and failed to produce typical blast lesions on rice leaves. In penetration assays with onion epidermal and rice leaf sheath epidermal cells, appressoria formed by the *mgset3*, *mgsnt1*, and *mghos2* deletion mutants were reduced in the efficiency of appressorial penetration and defective in the differentiation and growth of infectious hyphae. These mutants also were reduced in conidiation and vegetative growth but retained male fertility. In contrast, the *mghst1* and *mghos4* deletion mutants had no obvious defects in plant infection, fungal growth, and differentiation. These data suggested that the Set3 complex is conserved in *M. grisea* and play a critical role in plant penetration and infectious growth. Experiments to further confirm the interaction between members of the MgSet3 complex are in progress.

558. Light in the dark of invasive aspergillosis: Bioluminescent *Aspergillus fumigatus* as a tool for in vivo monitoring of pathogenesis

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Aspergillus fumigatus is a major pathogen of immunocompromised patients causing life-threatening invasive aspergillosis. Patients at risk suffer from different illnesses, but generally the function of effector cells from the innate immune system is impaired. Additionally, management of Graft-versus-Host Disease includes the immunosuppression by corticosteroids and therapy of leukaemia involves a phase of neutropenia. To monitor disease development under in vivo conditions, we constructed a bioluminescent *A. fumigatus* strain expressing the firefly luciferase under the control of the constitutively active *gpdA* promoter. In this strain the light emission correlated well with fungal biomass formation. To study the temporal and spatial resolution of pathogenesis, mice were immunosuppressed with corticosteroids and infected with the bioluminescent strain. A strong and ongoing bioluminescence was observed already 24 hours post infection, indicating a rapid germination of conidia, which was confirmed by histopathologic analyses. Therefore, the bioluminescent strain is a valuable tool to follow the development of invasive aspergillosis and can be used to study the effect of different immunosuppression regimens on the outcome of *A. fumigatus* pathogenesis under in vivo conditions.

559. Development of fluorescent protein-based biosensors for Ca^{2+} and pH to monitor physiological changes during *Arabidopsis thaliana-Fusarium oxysporum* interactions

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Both calcium and pH are important signal molecules that participate in the perception of many different types of external stimuli and control growth of both the plant and the pathogen. Changes in cytosolic free calcium ($[Ca^{2+}]_C$) can be evoked by various external stimuli and lead to physiological and/or developmental changes of the cell. The pH sensing-response signaling pathway also affects the activity of pathogenicity factors secreted by certain pathogens. To monitor real-time physiological changes during plant-pathogen interactions under different environmental conditions, we employed fluorescent Fluorescence Resonance Energy Transfer (FRET)-based Ca^{2+} sensors (Cameleon) and a pH sensitive biosensor. We successfully transformed two fungi, *Fusarium oxysporum*, *Magnaporthe oryzae*, and *Arabidopsis thaliana* with this FRET sensor and the pH biosensor to study Ca^{2+}/pH dynamics during their interactions. Time-lapse confocal imaging confirmed a tip high calcium gradient and pH changes in both fungal species. In addition, calcium responses in relation to key events in fungal growth such as branching, septum formation and cell-cell contact were observed. We also observed Ca^{2+}/pH changes during fungal growth on plants and response to other substrates. The combination of molecular genetics and molecular cytology tools with these biosensor tools should help us dissect the genetic, cellular, and biochemical basis of pathogen- plant interactions at the cellular and tissue levels.

560. A host dicer is required for antiviral defense and contributes to viral RNA recombination in *Cryphonectria parasitica*

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The disruption of one of two dicer genes, *dcl-2*, in the chestnut blight fungus *Cryphonectria parasitica* increased susceptibility to mycovirus infection. Virus- derived small interfering RNAs (vsRNAs) accumulated in hypovirus CHV1-EP713-infected wild-type and dicer gene *dcl-1* mutant *C. parasitica* strains, but not in hypovirus-infected *dcl-2* mutant and *dcl-1/dcl-2* double mutant strains. The accumulation of *dcl-2* gene transcripts increased significantly (12-20 fold) in response to mycovirus infection and increased even higher (~35 fold) after the infection by a hypovirus mutant Delta-p29, that lacks the RNA silencing suppressor p29.

Characterization of viral defective interfering RNAs (DI RNAs) in hypovirus infected *C. parasitica* revealed another important role of *dcl-2*. Defective RNAs accumulated in wild type and *dcl-1* mutant strains but not in *dcl-2* mutant, suggesting that *dcl-2* is required for the viral RNA recombination process involved the generation of viral DI RNAs. Studies with recombinant viral vectors indicate that *dcl-2* also contributes to the instability of foreign gene sequences in recombinant hypovirus genomes during viral RNA replication. These results provide the first evidence for a role of RNA silencing in viral RNA recombination.

561. Identification of a transcription factor regulating hyphal differentiation and growth in *Epichloe festucae*, a mutualistic symbiont of temperate grasses

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The fungal endophyte, *Epichloe festucae*, forms a symbiotic association with perennial ryegrass, *Lolium perenne*. In wild-type associations, *E. festucae* grows systemically in the intercellular spaces of the leaves as infrequently branched hyphae parallel to the leaf axis. *Agrobacterium tumefaciens* mediated T-DNA mutagenesis was used to identify genes of *E. festucae* responsible for maintaining the mutualistic association. The screen identified one mutant, Ag413, which causes severe stunting of the grass host, a host interaction phenotype very similar to that observed for a *noxA* deletion mutant (Tanaka et al., 2006). Sequence analysis of the genomic DNA flanking the T-DNA insertion in Ag413 showed that the T-DNA was inserted into a gene, designated *proA*, encoding a Zn(II)2Cys6 transcription factor. ProA has homology to Pro1/NosA, positive regulators of sexual development in other ascomycetes. Deletion analysis confirmed that stunting of the host plant is caused by disruption of *proA*. Homologues of *proA* were also deleted in the plant pathogens, *Magnaporthe oryzae* and *Fusarium oxysporum*, but no defect in pathogenicity was observed. Tanaka et al. (2006) Plant Cell 18, 1052-1066.

562. The transcription factor Con7, essential for the pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* on tomato plants.

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The study of transcription factors involved in the regulation of the morphologic development is essential for understanding the infection process carried out by fungal pathogens. Recently, the transcription factor Con7p has been described as a cental regulator of infection-related morphogenesis in the rice blast fungus *Magnaporthe grisea*. The *M. grisea* deficient strain was unable to form appressoria or to penetrate the rice plant leaves. The encoded protein regulates several genes important for morphogenesis and pathogenicity, including a chitin synthase, chitin binding proteins or an avenacinase-like encoding gene. Here we describe the targeted inactivation of the orthologue of *con7* gene in the soilborne pathogen *Fusarium oxysporum* f. sp. *lycopersici*, which causes vascular wilt disease on tomato plants. The predicted protein conserves highly similar motives like the nuclear localization signal, DNA binding motif and a coiled-coil region. Targeted disruption of the Fo*con*7 gene revealed its importance for fungal morphogenesis and the infection process. Null mutants showed alterations in polar growth and mycelial branching, and did not induce disease symptoms on tomato plants. Identification of the genes regulated by the Con7 transcription factor in *F. oxysporum* is under investigation since this would improve the understanding of the infective process.

563. *Trichophyton rubrum* expressed genes during ex vivo infection of skin and nails.

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Host-fungal interactions are dynamic and complex, and in order to identify new fungal targets and develop more effective antifungal therapies, it is important to understand cellular and molecular mechanisms involved in the infection process. The most prevalent fungal infections in immunocompetent individuals are the dermatophytosis, mainly caused by *Trichophyton rubrum*. For a better understanding of *T. rubrum*- host interactions, we developed an ex vivo skin infection system and used the suppressive subtraction hybridization approach to identify *T. rubrum* genes involved in fungal-skin interaction. Variation in expression levels of candidate genes during skin infection, and also after nail and keratin growth, was analyzed on a set of selected genes. The identification of pathogen genes expressed during infection is a crucial step in exploring the molecular mechanisms contributing to the pathogenicity of *T. rubrum*. Moreover, the differentially expressed genes identified here are potential targets for further molecular studies of virulence, using an experimental infection model. Financial Support: CNPq, CAPES, FAPESP, FAEPA

564. Both ATP citrate lyase subunits, ACLA and ACLB, are crucial components for sexual and asexual development in *Gibberella zeae*

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Gibberella zeae (anamorph : *Fusarium graminearum*) that causes Fusarium head blight (FHB) on wheat and barley is the major cause for economic loss in cereal crops. In addition, mycotoxins that remained on the infected cereals can threaten to human and animal health. To characterize the genes responsible for fungal virulence, we used Restriction Enzyme-Mediated Integration (REMI) for random mutagenesis and found a mutant, Z39P186, that reduced sexual development and virulence. Vector insertion site was 36bp upstream of FGSG_06039.3 which is similar to ATP citrate lyase subunit A (ACLA) of *Sodaria macrospora*. Another ACL subunit, ATP citrate synthase (ACLB), was located in 3.4 kb upstream of ACLA in reverse direction. ACL is responsible for the production of cytosolic acetyl CoA which is precursor for the biosynthesis of sterols and lipids, by cleaving citrate transported from mitochondria. To elucidate the functions of each subunit, we made deletion mutants, delta-*GzACLA*, delta-*GzACLB* and delta-*GzACLA* delta-*GzACLB*, by using gene replacement strategy. Although cytosolic acetyl CoA is essential for survival, these mutants was not lethal but exhibited pleiotropic changes including reduction of mycelial growth and virulence, abnormal shape of macroconidia and abolished sexual reproduction. These results indicate other pathways for cytosolic acetyl CoA production, like acetyl CoA synthetase (AS) that exist three homologs in genome of *G. zeae*, could compensate enough for the vegetative growth to some degree but not for the sexual and asexual developments and virulence. Hyphal growth on different carbon sources showed that expression or activity of ACL subunits were dependant on carbon sources. Conclusively, ACL-dependant cytosolic acetyl CoA is crucial for both sexual and asexual development and takes it's roles in different life stages.

565. Naturally-occurring variation in virulence in *Aspergillus nidulans* .

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Several species within the genus *Aspergillus* can cause fatal infections in immunocompromised individuals including transplant recipients and patients with chronic granulomatous disease. The long-term aim of this study is to identify genes associated with virulence using quantitative trait loci (QTL) mapping. As a first step towards this goal, we are measuring natural variation in growth and virulence-related traits within *A. nidulans*, a pre-requisite for QTL mapping. The ability to *A. nidulans* to overcome iron limitation is considered a virulence factor because free iron concentrations are very low within the host. Radial growth measurements, taken for four consecutive days on iron-limited Neiland's agar medium, were used to determine variation in growth in parental and recombinant strains (isolated from a cross between the parental strains). Significant variation in the rate and extent of growth was observed among parental and recombinant strains. The strains are also being tested in *Galleria mellonella* larvae (wax moth) to observe variation in virulence *in vivo*. These findings will provide insight into variation in growth and virulence within *Aspergillus* species and will ultimately help to identify the genes responsible for that variation.

566. The ER chaperone MoLHS1 is involved in asexual development and rice infection by the blast fungus *Magnaporthe oryzae*

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In planta secretion of fungal pathogen proteins, including effectors destined for the plant cell cytoplasm, is critical for disease and yet little is known about the secretion mechanisms used by these pathogens. The endoplasmic reticulum (ER) is a highly specialized eukaryotic organelle involved primarily in protein secretion and in certain types of protein modification. To determine if normal ER function is crucial for fungal pathogenicity, genes encoding proteins homologous to Lhs1p and Kar2p, members of the heat shock protein 70 (Hsp70) family in *Saccharomyces cerevisiae*, were cloned and characterized from the rice blast fungus. Like their yeast counterparts, both MoLHS1 and MoKAR2 proteins localized in the ER, and they function in an Unfolded Protein Response (UPR) similar to the yeast UPR. Mutants produced by disruption of *MoLHS1* were viable and showed a defect in the translocation of proteins across the ER membrane and reduced activities of extracellular enzymes. The *delta-molhs1* mutant was severely impaired in conidiation, and in both penetration and biotrophic invasion in susceptible rice plants. The mutant also had defects in the induction of the *Pi-ta* resistance gene-mediated hypersensitive response, and in the accumulation of secreted fluorescent effector proteins in Biotrophic Interfacial Complexes. Our results suggest that proper processing of secreted proteins, including effectors, by chaperones in the ER is requisite for successful disease development and for determining host- pathogen compatibility via the gene-for-gene interaction.

567. Ca2+/ calcineurin-dependent signal transduction is required for differentiation and virulence in *Botrytis cinerea*

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The transcription factor BcCRZ1 induces gene expression after its dephosphorylation by the Ca2+/calmodulin-dependent phosphatase calcineurin and subsequent translocation into the nucleus. Deletion of *bccrz1* affects vegetative growth and differentiation (less conidia, no sclerotia), cell wall and membrane integrity as well as the resistance to different stressors. While BcCRZ1 is almost dispensable for conidium-derived infection, hyphae of *bccrz1* mutants are significantly impaired in the ability to penetrate the intact host surface. Interestingly, Mg2+ supplementation restores growth rate, conidiation, and penetration, but has no impact on sclerotium formation and the hypersensitivity to oxidative stress. In accordance with the model that postulates BcCRZ1 activation when cytosolic Ca2+ levels are increased, high Ca2+ concentrations are lethal for *bccrz1* mutants, even in presence of Mg2+, as the appropriate signal transduction via BcCRZ1 is blocked. Contrary to our previous expectation that the deletion of calcineurin is lethal, we recently succeeded with the isolation of calcineurin A (*bccnA*) null mutants. Mutants are severely impaired in growth and virulence: strains grow very slow, are only viable on grape juice medium and are non-pathogenic. The fact that the *bccnA* deletion phenotype is more severe than that of *bccrz1* mutants, corroborates our hypothesis that additional downstream targets of calcineurin exist. To gain new insights into Ca2+/calcineurin-dependent signal transduction, we generated mutants displaying modified calcineurin activities and expression levels of *bccrz1*.

568. *Plasmodium falciparum* and *Hyaloperonospora parasitica* effector translocation motifs are functional in *Phytophthora infestans*

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Like bacteria and fungi, the potato blight pathogen *Phytophthora infestans* translocates effector proteins into host plant cells during infection. In *Phytophthora* this process depends on a short conserved amino acid sequence (RxLR) located near the signal peptide of hundreds of secreted proteins. This motif is similar to the host cell targeting-signal found in virulence proteins from the malaria parasite *Plasmodium falciparum*. A recent study showed that the RxLR motif from AVR3a was sufficient to export the green fluorescent protein (GFP) from *P. falciparum* to the erythrocyte, suggesting a conserved mechanism to deliver effector/virulence proteins into host cells. We have used the AVR3a-R3a interaction as a reporter for translocation in *P. infestans* transformants and replaced the AVR3a RxLR-EER motifs with the RxLR- EER, RxLR only, or RxLxE/D/Q from a related oomycete or *P. falciparum*, respectively. Transformation of a virulent *P. infestans* isolate with the various *Avr3a* constructs yielded avirulent transformants, implying that the alternative sequence is functionally similar to the native RxLR-EER.

569. The Gbeta-gamma dimer and the Galpha subunit BCG1 have overlapping and distinct functions in the plant pathogen *Botrytis cinerea*
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Several signalling cascades have been shown to be involved in the *B. cinerea*- host interaction: MAPK cascades are essential for penetrating the host tissue, while heterotrimeric G proteins and the cAMP pathway are required for colonization and subsequent reproduction. The Galpha subunit BCG1 regulates the transition from primary to spreading infections via affecting (at least) two signalling cascades: the cAMP cascade and the Ca2+/calcineurin pathway. We studied the role of the beta-gamma dimer in regulation of both signalling pathways by deleting the gene encoding the Gbeta subunit BCGB1, and by generation of mutants expressing dominant-active or dominant- negative BCG1. *bcgb1* mutants displayed increased aerial mycelium formation, produced almost no conidia, were unable to develop sclerotia, were retarded in the infection process and unable to cause soft rot. The increased cellular cAMP levels of *bcgb1* strains and the similarity to DAbcg1 mutants suggest that BCGB1 is a negative regulator of cAMP-dependent signalling. While only BCG1 functions in regulation of production of oxalic acid, proteases and phytotoxins in a cAMP-independent manner, both subunits BCG1 and BCGB1 are involved in regulation of conidial germination, germ tube differentiation and penetration. Conidia of *bcg1* and *bcgb1* mutants displayed inappropriate germination rates in water (without inductive signals), and formed elongated, less branched germ tubes on all tested surfaces. Hence, a signalling function of the inactive heterotrimeric G protein composed of BCG1 and the beta-gamma dimer in regulation of germination is proposed.

570. Towards identification of effector proteins in the lettuce downy mildew pathogen *Bremia lactucae*
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Bremia lactucae is an obligate biotrophic pathogen of lettuce (*Lactuca sativa*). Like other downy mildews and *Phytophthora* species it belongs to the oomycetes (kingdom Stramenopiles). During infection Bremia grows intercellular hyphae and forms haustoria in host cells. In lettuce cultivation Bremia is mainly controlled by dominant resistance genes that are rapidly overcome by new isolates. Durable resistance is more desirable than ever, as Bremia is also becoming increasingly resistant to fungicides. The aim of this project is to identify Bremia effector proteins and to study their role in the infection process and in disease susceptibility. We have generated 5' cDNA and random- primed normalised cDNA from both Bremia spores and heavily infected lettuce leaves for 454-sequencing. An initial run yielded 46699 assemblies with an average length of 346 base pairs. To increase coverage and lengthen these assemblies additional 454- sequencing is being performed. Potential effectors will be selected from assembled EST sequences by the presence of predicted signal peptides, RXLR-motifs and other known effector characteristics. Currently we are assessing bio-assays to test for disease- promoting and defence suppressing activities of the potential effectors identified by sequencing. We will report on the preliminary analysis of the Bremia transcriptome. The knowledge gained from this project will be used to screen for lettuce lines that are insensitive to the action of important effector proteins that can then be deployed for resistance breeding.

571. A novel fungal gene designated SnTox3 encodes a host-selective toxin important in the wheat-*Stagonospora nodorum* interaction
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The necrotrophic fungus *Stagonospora nodorum*, causal agent of S. nodorum blotch of wheat has been shown to produce multiple proteinaceous host-selective toxins (HSTs) that are important factors in disease. Here, we report the molecular cloning and functional characterization of the toxin-producing gene *SnTox3*. *SnTox3* is a 693bp intron free gene with little obvious homology to any other known genes. Using heterologous expression in *Pichia pastoris* this gene was shown to encode for the HST SnTox3. Transformation of *SnTox3* into an avirulent *S. nodorum* isolate produced a strain with virulence on wheat lines containing *Snn3*, the SnTox3 sensitivity gene. Gene disruption showed that mutants without intact *SnTox3* are deficient in the production of SnTox3 as well as avirulent on the *Snn3* (SnTox3 sensitivity) wheat differential line BG220. The SnTox3 protein contains a predicted signal sequence of 20 amino acids. Site directed mutagenesis within the protein suggest that a C-terminal region is critical for biological activity of the toxin. Genetic diversity analysis of a worldwide *S. nodorum* collection revealed that *SnTox3* was present in approximately 50% of the isolates evaluated and appears in 10 nucleotide haplotypes. The 10 haplotypes result in four functional amino acid haplotypes with two being rare and two being highly predominant. This research strengthens the notion that the *S. nodorum*-wheat pathosystem is largely based on multiple host-toxin interactions that follow an inverse gene-for-gene scenario.

572. Comparative genomics of the fungal plant pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum*
Botrytis/Sclerotinia genomes consortium at http://urgi.versailles.inra.fr/projects/Botrytis/, M Dickman[1] and M-H Lebrun[2]. [1] IPGB, TAMU, Texas, USA, [2] BIOGER, INRA, Versailles, France

Genomes of *B. cinerea* strains T4 (Génoscope/URGI) and B05.10 (Syngenta/Broad Institute) as well as *S. sclerotiorum* strain 1980 (Broad Institute), contain at least 13.000 genes. These genomes displayed a common set of 10,500 genes with a high degree of sequence similarity (85 % average protein sequence identity) and frequently found in syntenic blocks. Similar genes encoding plant cell wall degrading enzymes were found in both species consistent with their capacity to degrade pectin and cellulose found in their host plants cell walls (dicots, fruits). However, these two species significantly differ in their repetitive DNA content, as *S. sclerotiorum* has more transposons (8 % of the genome) than *B. cinerea* (1 %). This difference is related to the expansion of different class of transposable elements that have been subjected to RIP in both species, although to a greater extent in *B. cinerea*. Important differences in genes encoding secondary metabolism enzymes, transcription factors, membrane transporters as well as mating-type loci were also identified between these two species, while other genes such as those encoding secreted proteins and metabolic enzymes were generally similar. These findings suggest that these two species, while having a number of similarities, have evolved different strategies as successful necrotrophic plant pathogens.

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Education

573. How do we prepare future faculty?
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At Indiana University, a number of faculty have had the good fortune to participate in IU's Freshman Learning Project (FLP; http://www.iub.edu/~flp/), an intensive, two-week seminar in which faculty learn about a number of issues that affect student learning. Each participant also chooses a "bottleneck," which is a concept or thinking process that students find difficult. Participants then design classroom lessons to help students through those bottlenecks, practice these lessons on one another, and then implement them in their own courses.
I now lead a "Mentored Teaching" graduate seminar, which is meant as an FLP-like experience for graduate students. The course is based upon what Biology faculty found most valuable in their FLP experiences and on the advice of the teaching pros.
In this course, students: read about and discuss issues related to teaching in general and to science and science teaching; make field trips to observe effective teachers, particularly those engaged in "decoding the disciplines" methods of helping students learn to do disciplinary thinking; interview teachers of introductory courses about bottlenecks; choose a bottleneck and design a lesson to address it; practice the bottleneck lesson on peers; "road test" the lesson on a group of actual undergraduates; and write regular reflections on the readings, class meetings, and the bottleneck lessons.
In my poster and talk I will describe the course and also present topics and resources we have found the most useful so far.

574. DelsGate a robust deletion method used as a tool for undergraduate teaching in fungal genomics.
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A recently published method (Garcia-Pedrajas et al., FGB 2008) called DelsGate for the production of constructs for gene deletion in *Ustilago maydis* has been used as part of an upper division undergraduate laboratory course in applied biotechnology at The University of Georgia for the past three years. Students are each assigned a unique gene of research interest. Students are trained in accessing and downloading their gene locus from the genome sequence at the MIPS site MUMDB. They design primers for precise deletion of their gene, carry out the PCR reactions and check their construct for appropriate structure. Finally, they transform the fungus and analyze transformants for deletion of their gene of interest. Success rates are high due to the straight-forward and robust nature of the DelsGate approach. Students each compile a report of their experimentation including an analysis of potential gene function, genomic context of their gene and their deletion plan and experimental results and interpretations. The deletion mutants produced by class participants are fed back into the research lab and are further analyzed by laboratory personnel. Student evaluations suggest that the individual nature of this approach is appreciated for its real life research exposure.

575. Neurospora genetics and genomics summer research institute: an introduction to research.
Gloria E. Turner & Richard L. Weiss. University of California, Los Angeles

Phenotypic analysis of gene deletion strains was used to introduce college sophomores to the research process. When they entered the program, genetics, genomics and the model organism, *Neurospora crassa* were unknowns. At the completion they were well versed in these subjects as well as skilled in several basic microbiological laboratory techniques. We recruited students from UCLA and local Community Colleges that were participating in outreach programs for underrepresented minorities and first generation college students. A full-time nine-week program was developed focusing on the five assays that comprised the basic phenotypic analysis. These assays examined the fundamental biological consequences resulting from the loss of a single gene in a knockout (KO) mutant produced by the Neurospora PO1 project. The data generated by our program is transferred to the Broad Institute at Harvard/MIT. A data entry form designed to capture each mutant analysis aided in the information transfer. The students attended a scientific writing class, seminars given by prominent scientists, Excel and Power Point workshops as well as an introduction to NCBI. An independent research project was required of all students allowing them to generate a scientific question and research methodology. Each student performed their research and presented the results at the close of the program. These efforts have resulted in 900 KO phenotypes available on the *Neurospora crassa* database website at the Broad Institute. Equally important, several program participants are now pursuing masters and doctoral degrees in the sciences.

576. Plant pathology vs. medical mycology: battle of the fungi.
Volk, Thomas J., Curland, Rebecca , and Jarvis, Elisabeth. Department of Biology, University of Wisconsin-La Crosse, La Crosse, WI 54601. volk.thom@uwlax.edu http://TomVolkFungi.net

Although humans and plants are clearly different disease hosts, the study of fungal diseases in plants and animals share many more characteristics than are generally acknowledged. As one of the few departments to teach both Plant Pathology and Medical Mycology courses, we have a unique perspective. Plants and humans are similar in the taxa of fungal pathogens (Koch's postulates!), modes of infection, and how pathogens travel through the organism. Plants and humans share broad categories of infections such as superficial diseases of the epidermis, traumatic implantation, and systemic pathogens. Conditions such as stress / weakened defense system or high heat and humidity can favor fungal growth in both groups. However, given the dissimilarity between the two hosts, the signs, symptoms, host defenses, and treatment methods differ greatly. Where is the disease triangle in medical mycology? Most fungal plant pathogens are specific to one host species, but can easily spread between individuals. In contrast, almost all fungal human diseases affect other mammals, but are typically not contagious. Human mycoses are typically more difficult to treat because of similarities between fungal and animal cells and their close phylogenetic relationship. The main purpose of this poster is to show that Plant Pathology and Medical Mycology are not so different after all, and, despite different terminology for similar processes and structures, both groups have much to learn from each other.

Other Topics

577. Birch wood volatiles induce a chemotropic response in *Schizophyllum commune*

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The saprotrophic fungus *Schizophyllum commune* can be found all over the world on felled hardwoods. The life-cycle of *S. commune* starts with the germination of a spore into a monokaryotic mycelium. Two monokaryons with different A and B mating- type loci can fuse resulting in a dikaryon, which forms sporulating fruiting bodies under appropriate environmental conditions. Birch wood induces a positive chemotropic growth response in mono- and dikaryotic substrate hyphae. Formation of aerial hyphae is also stimulated, however, much stronger in monokaryons. Aerial hyphae grow specifically towards birch wood, but only when it is not already being colonized by another mycelium. The active component(s) is extracted from birch wood with methanol. In the near future we are planning to separate this extract on a C18 column and to assay the different fractions on activity. Headspace GC/MS will be used to identify the active volatile. Taken together, these data indicate that in nature colonization of new substrates by *S. commune* is facilitated by chemotropic growth of aerial hyphae across air gaps in the wood. Monokaryons may more efficiently do so since aerial hyphae formation is much more pronounced in these strains than in dikaryons.

578. Study on genes related to lignocellulose degradation in *Lentinula edodes*

Iris S.W. Kwok, Winnie W.Y. Chum, Tommy C.H. Au and H.S. Kwan Department of Biology, Chinese University of Hong Kong, HKSAR, China

During vegetative growth, *Lentinula edodes* degrades lignocellulose efficiently by secreting lignocellulolytic enzymes. We identified these lignocellulolytic enzyme-coding genes by large-scale sequencing of mycelial cDNA. Lignin degradation involves the formation of reactive oxygen species (ROS). We also studied the antioxidative activity of mycelia by qRT-PCR analysis on expressions of genes related to antioxidative response. More than 6000 ESTs were obtained from large-scale sequencing-by-synthesis of cDNA from lignocellulose-grown mycelia. To obtain more complete analysis on the expression profile of these lignocellulolytic enzymes, those 6000 ESTs, together with thousands of *L. edodes* ESTs available in NCBI, were subjected to in-house BLAST against FOLy (Fungal Oxidative Lignin enzymes) and CAZy (Carbohydrate-Active enZymes) databases. About one thousand ESTs could be classified into different FOLy and CAZy groups. Eight genes related to antioxidative response, including catalase (Cat) and superoxide dismutase (SOD), have been identified from those 6000 ESTs according to Gene Ontology. Quantitative RT-PCR analysis revealed the transcription levels of these genes were lower in lignocellulose-grown mycelium, compared to that in non-lignocellulose- grown mycelia. Cat and SOD also showed lower activities in lignocellulose-grown mycelium. We propose that during lignin degradation, mycelia may encounter lower oxidative stress despite the production of ROS due to the scavenging of them by lignin.

579. Isolation and characterization of an Aspergillus niger insertional mutant exhibiting elevated level of heterologous laccase secretion.

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To isolate *Aspergillus niger* mutants with improved expression level of extracellular, heterologous laccase, we transformed *A. niger* strain GICC2773 with a plasmid pMW1 carrying a hygromycin resistance gene to generate a library of randomly insertional mutants. Out of >8000 transformants screened, we identified 8 mutants with 25-49 % increases in extracellular laccase production. To characterize the locus of the plasmid insertion, we developed a modified TAIL-PCR assay termed SM-TAIL-PCR to amplify target genomic DNA and we have identified the insertion site in a mutant strain 16H2. To confirm the mutation phenotype we recreated a mutation strain by targeted disruption (strain ?16H2). Compared to the wild type strain, the strain ?16H2 produced higher level of extracellular laccase, suggesting that the mutation in strain 16H2 affected the expression/secretion of the laccase. DNA sequence analysis did not identify any large ORF at the insertion site. We analyzed the two flanking genes ptrA and ssoA and showed that the only the transcript of the ptrA gene was reduced. We hypothesize that reduction of the ptrA transcription might contribute to the regulation of the A. niger secretion pathways.

580. Efficient *Agrobacterium*-Mediated Transformation (AMT) in *Aspergillus fumigatus* for genome-wide functional studies.

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Aspergillus fumigatus is one of the most common fungal pathogens affecting neutropenic patients. Lack of defined virulence traits and lack of efficient tools to generate large number of easily identifiable mutants hinders the investigation of the molecular mechanisms important for its pathogenesis. Insertional mutagenesis via *Agrobacterium*-mediated transformation (AMT) is the method of choice as a forward genetic approach because it allows for rapid identification of the mutated gene that yields a particular phenotype. However, the major limitation with AMT in *A. fumigatus* is low efficiency. Here we modified the AMT protocol in transforming *A. fumigatus*, which not only increased transformation efficiency by ~30 fold, but also afforded a simplified subsequent procedure to obtain uni-nuclear derived transformants. To validify our modifications, a mutant collection generated via our method was screened for defects in conidiation. Mutants that conidiated poorly and those that were totally barren were isolated. Novel genes as well as genes known to be involved in the conidiation pathways (e.g. BrlA) have been recovered. Therefore, the AMT protocol developed here is highly efficient to be used in any desired *A. fumigatus* background for genome- wide functional studies. The modifications can also be extended to AMT transformation in other filamentous fungal systems and will greatly facilitate further genetic studies in these organisms given the ever increasing sequenced fungal genomes.

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581. Upregulated fungal genes in the early developmental stages of lichen symbiosis.

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One fifth of all known fungi are obligatory symbiotic partners with green algae, cyanobacteria, or both. An emergent property of this symbiosis is the formation of a lichen thallus. Although lichens are classic in illustrating mutualistic relationships, slow growth and genetic intractability have limited our progress in understanding the molecular basis to lichen development. Using the symbiosis between the fungus *Cladonia grayi* and the green alga *Asterochloris* sp., we have investigated differentially expressed genes in early thallus development. We used Suppression Subtractive Hybridization to find up-regulated genes in the early developmental stages between *C. grayi* and *Asterochloris* sp. in *in vitro* resynthesis. Among hundreds of identified gene fragments we chose candidate fungal genes to confirm differential gene expression using quantitative PCR. Our results parallel those seen in the mutualistic interactions between mycorrhizal fungi and their hosts: The development of both mycorrhizae and lichens appear to involve epistatic interactions between genes showing slight quantitative variation. The results of these experiments will be discussed and put into the context of putative roles in the lichens symbiosis and fungal symbiotrophy in general.

582. Selection of microbial production hosts for real-life feedstock utilization

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Alternative lower-cost substrate feedstocks include lignocellulosic hydrolysates and waste glycerol from biodiesel production. These "real-life" feedstocks can, however, contain mixtures of different fermentable sugars, high salt concentrations as well as specific compounds that strongly inhibit microbial growth, depending on the preceding feedstock pretreatment process. Wild-type strains of six industrially relevant microbial production hosts, including *Aspergillus niger* and *Trichoderma reesei*, 2 yeasts and 2 bacteria, were evaluated for their performance on lignocellulosic feedstocks and various other characteristics relevant for crude feedstock utilisation. The renewable feedstocks tested included corn stover, wheat straw, bagasse and willow wood and waste glycerol. Large differences in the overall performance of the six tested microbial production hosts were observed. In an overall scoring *Aspergillus niger* and *Pichia stipitis* were found to perform the best.

583. Production of full length antibody chains in *Chrysosporium*

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Filamentous fungi are widely used for enzyme production for a wide variety of uses – for example food, feed, textile, paper and pulp, fuels and chemicals, detergents - due to the development of extremely productive strains and production processes. Levels in the range of 100 grams protein per liter have been reported, repeatedly. Therefore, these organisms have also been considered for the production of pharmaceutical proteins. In the pharmaceutical industry, the main production platforms are E. coli and mammalian cell lines. However, for those pharmaceutical proteins for which high yields and low production costs are important, filamentous fungi could provide a viable alternative. We have explored the use of a highly productive fungal production platform *Chrysosporium* (C1) for the production of a very versatile class of pharmaceutical proteins, i.e., antibody molecules. Antibody molecules and molecules carrying antibody domains are currently the largest and fastest-growing class of biopharmaceuticals. Production of functional full-length human monoclonal antibodies has been accomplished using highly productive low protease mutant *Chrysosporium* host strains. High level expression was achieved using a glucoamylase-carrier approach, and recombinant strains expressing both heavy and light chains were obtained. Heterodimeric antibody molecules were formed efficiently, allowing simple purification of the protein from the culture fluid using protein A. Cell-based bio-assays performed on the culture supernatant and the purified samples revealed almost complete bioactivity.

584. Cross species yeast two-hybrid analyses reveal interaction partners of the developmental proteins PRO22 and PRO40 from *Sordaria macrospora*

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The formation of fruiting bodies in filamentous fungi is a multicellular differentiation process and controlled by many developmentally regulated genes. The filamentous ascomycete *S. macrospora* represents an excellent model system for cell differentiation during fruiting body development, because this homothallic fungus is self- fertile. For *S. macrospora*, several developmental mutants have been described. Though these mutants can build young protoperithecia, they are not able to form mature perithecia. These mutants, include the mutants pro22 and pro40. By means of forced- heterokaryon tests using auxotrophic strains and fluorescence microscopy using nuclear labeled strains it was demonstrated that these mutants are restricted in hyphal anastomosis in homozygous crosses. Due to the close relationship between *S. macrospora* and *Neurospora crassa*, a cDNA library of *N. crassa* was used for cross species yeast two- hybrid analyses to identify putative interaction partners of PRO22 and PRO40. Our aim is to verify these putative interaction partners *in vitro* and *in vivo* and to identify a regulatory network involved in fruiting body formation.

585. Novel gene discovery and molecular markers development of shiitake mushroom *Lentinula edodes* by high-throughput sequencing.
M.C. Wong, C.H. Au, Winnie W.Y. Chum, P.Y. Yip, Iris S.W. Kwok, Patrick T.W. Law, Y. He, H.W. Cheng, H.S. Kwan. The Chinese University of Hong Kong, HKSAR, China.

Shiitake mushroom *Lentinula edodes* is a popular edible mushroom of high economic values. Next generation sequencing and bioinformatics tools were employed for the genomic analysis of *L. edodes* to reveal novel genes and to develop molecular markers for strains identification. A genome survey sequencing (GSS) of dikaryotic *L. edodes* L54 were performed using 454 GS-20 (Roche). A total of 353,030 reads (total length 35Mbp) were assembled into 31,000 contigs. To identify novel genes, the GSS data, the 12,000 unpublished in-house cDNA contigs and the 12,210 publicly available expressed sequence tag (EST) sequences in NCBI dbEST were searched using BLASTX against five other sequenced basidiomycetes. To develop simple sequence repeat (SSR) markers, 31,000 GSS contigs and the existing 24,210 EST sequences were used to search for SSR motifs, resulting in 587 sequences, containing at least 1 SSR motifs per sequence. A number of these SSR motifs were confirmed by cloning and sequencing. Their degrees of polymorphism across strains are being examined. To construct a draft genome sequence of *L. edodes*, as well as revealing more genes and SSRs, whole genome sequencing of monokaryotic strain L54- A, by both paired-end and shotgun approaches is ongoing using 454 GS-FLX (Roche).

586. *Phycomyces blakesleeanus* and *Rhizopus oryzae* show significant differences in carbon utilization compared to ascomycete and basidiomycete fungi.
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In nature, a large diversity of plant-based polysaccharides is present. Fungi degrade plant polysaccharides by the production of a broad range of enzymes. The increasing number of fungal genome sequences gives the opportunity to compare the potential of fungi to degrade plant polysaccharides. Previously, CAZy annotation of fungal genomes from basidiomycete and ascomycete fungi were shown to correlate with growth profiles on different carbon sources. The availability of two zygomycete genome sequences, enables us to expand this study into this class of fungi. In this study we compared the genomic potential and growth profiles of *P. blakesleeanus* and *R. oryzae*. We focused on enzyme families that are similar between the two zygomycetes but different in other fungi. Genome annotation is linked to growth on 36 carbon sources including crude substrates, mono-, oligo-, and polysaccharides. Strong correlations were found between growth and presence of relevant functions in the genome, while no growth correlated with absence of relevant functions. Moreover, both the genomic potential and growth profile of *R. oryzae* and *P. blakesleeanus* were highly similar, but significantly different from other fungi, suggesting that zygomycetes have a distinct role or place in their natural ecosystems.

587. AP-PCR and high-throughput sequencing for *Lentinula edodes* strain differentiation
Y. He, M.C. Wong, Winnie W.Y. Chum, P.Y. Yip, T.W. Law and H.S. Kwan The Chinese University of Hong Kong, HKSAR, China

Arbitrarily primed-polymerase chain reaction (AP-PCR) was performed to differentiate *Lentinula edodes* strains from China. Twenty 25-base primers were used to generate AP-PCR fingerprints among 10 *L. edodes* strains. Eight primers produced polymorphic bandings in all 10 strains tested. Three strains had unique DNA fingerprints, whereas *L. edodes* strain 25 exhibited distinct polymorphic profiles with all primers tested. Molecular markers obtained from AP-PCR assay can be used to differentiate strains of *L. edodes* and have potential applications in mushroom breeding and strain improvement programs. To further develop specific sequence characterized amplified region (SCAR) markers, strain-specific bands from the fingerprints are usually cloned, screened and sequenced. Specific primers are designed according to the sequences and tested whether they can generate SCAR markers. The development of SCAR markers in this way is laborious and inefficient. We combined AP-PCR with high-throughput sequencing to generated SCAR markers. The AP-PCR products of dikaryotic strains were sequenced using 454 GS-FLX (Roche). By comparing the DNA profiles of different strains, large number of strain- specific sequences can be identified. Primer pairs that specifically amplify the sequences were designed, tested and developed to SCAR markers.

588. Investigating pleuromutilin producing fungi
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Retapamulin is the active ingredient of a new antibiotic that has recently been approved for topical application in humans. The antibiotic is derived from pleuromutulin, a tricyclic diterpene that has been used in veterinary medicine for more than 20 years as Tiamulin or Valnemulin. The compound is produced by fermentation of the basidiomycete *Clitopilus passeckerianus*, but the literature also reports other fungi as being producers of this class of antibiotic. In this poster we will present the results of our investigations into the taxonomic status of these fungi, with particular emphasis on those that produce pleuromutulin. In addition we have also managed to generate mature fruiting bodies of this fungus with viable basidiospores. We will present initial analysis of the investigations into the classical genetics of this basidiomycete.

589. *Ustilago maydis* disease severity in maize is reduced by interactions with endophytic *Fusarium verticillioides*
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Plant associated-organisms form diverse communities in which they compete for limited resources or cooperate to exploit host nutrition. Previous studies have shown that these interactions are ubiquitous and that the outcome for host and microbial fitness is variable, depending on both biotic and abiotic factors. We used endophytic isolates of *Fusarium verticillioides* (Sacc.) Nirenberg and the corn smut pathogen, *Ustilago maydis* DC (Corda) on maize to understand how this endophytic fungus of maize interacts with *U. maydis* and affects the plant host¡¯s growth. Two different endophytic isolates of *F. verticillioides* were inoculated onto maize seedlings before, after or at the same time as *U. maydis* was inoculated, and subsequent *U. maydis* disease severity and plant growth were assessed. Simultaneous coinoculation of *F. verticillioides* with *U. maydis* had the greatest effects, significantly reducing the proportion of severely diseased plants and increasing host growth compared to *U. maydis* only inoculated controls. Together, our results suggest that *F. verticillioides* alone does not have significant direct effects on maize plant growth and direct interactions between these two fungi is the primary mechanism affecting *U. maydis* disease development and plant growth.

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590. Mating-Type genes in the asexual beta-lactam antibiotic producer *Penicillium chrysogenum*

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Eighty years ago, Alexander Fleming discovered an anti-bacterial activity in the asexual mold Penicillium. Later this original "Fleming strain" was replaced by an overproducing *P. chrysogenum* isolate still used for industrial penicillin production today. Using a heterologous PCR approach, we identified the sex genes and demonstrated that these strains are of opposite mating types. RT-PCR analyses showed that mating-type genes are expressed and suggest that *P. chrysogenum* has the potential to reproduce sexually. These findings prompted us to search for homologs of pheromone and pheromone receptor genes that function in mating and pheromone signaling in sexual reproducing filamentous fungi. Indeed, a screen of a cDNA library led to the isolation of transcriptionally expressed pheromone and pheromone receptor genes in strains of both mating types. The results of our transcriptional expression data suggest the existence of a heterothallic sexual cycle in *P. chrysogenum* [1]. To further test the functionality of the mating-type genes in this asexual fungus, we have generated deletion strains for further analysis. [1] Hoff B, Pöggeler S, Kück U (2008) Eukaryot Cell 7: 465-470

591. Functional analysis of an ectomycorrhizal fungus *Tricholoma vaccinum* aldehyde dehydrogenase.

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The mechanisms, especially at molecular level, of the symbiotic association between the basidiomycete ectomycorrhizal fungus *Tricholoma vaccinum* and its compatible host plant, spruce are largely unknown. As a first step to unravel the molecular basis of the interaction between the plant and fungal partners in this system, RNA fingerprinting was carried out to identify *T. vaccinum* genes that are specifically expressed during ectomycorrhizal interaction. An aldehyde dehydrogenase (*aldh*), among other genes, was identified by this effort. The possible function of *aldh* in ectomycorrhiza would be related to plant defense by detoxifying aldehydes and alcohols that accumulate when a plant is exposed to stress or production of indole-3-acetic acid (IAA), which has been controversially observed and discussed to be involved in ectomycorrhizal development. Using PCR methods, the full-length *aldh* was cloned. The gene has an ORF of 2502 bp, interrupted by 16 introns, and encodes a predicted protein of 514 aa. For functional characterization, the gene was overexpressed in *T. vaccinum*, and the transformants are now being tested. *In vitro* studies with IAA showed that the phytohormone increases the radial mycelial growth and hyphal branching of *T. vaccinum*, an observation that would probably indicate increased mycorrhization efficiency.

592. Genetic linkage map and STS markers for breeding of a sporeless strain in oyster mushroom, *Pleurotus pulmonarius*.

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In the cultivation of edible mushrooms, including *Pleurotus pulmonarius*, the enormous number of spores produced by fruiting bodies cause several serious problems such as respiratory allergies among mushroom growers and damage to mushroom cultivation facilities. Sporulation-deficient (sporeless) mutants are useful for preventing the above-mentioned problems in mushroom cultivation. To gather genetic data and develop the efficient detection system for sporeless trait of *P. pulmonarius*, we constructed a genetic linkage map and identified markers that closely linked to the sporeless trait by AFLP analysis and bulked segregant analysis based-AFLP (BSA) analysis using sporeless mutant (TMIC-30058) of this mushroom. The genetic linkage map comprised twelve linkage groups that have a total length of 971.1 cM with an average marker interval of 5.2 cM and the nearest marker to the sporeless locus was located 1.4 cM away. We also identified a total of 20 markers linked to the locus of sporeless mutation by BSA analysis. Tightly linked 18 markers were converted to sequence tagged sites (STS) markers. It was indicated that 2 STS primer pairs, SD192 and SD296, enable marker aided selection as useful STS markers in the breeding of a sporeless strain in *P. pulmonarius*. [Supported by Japan Society for the Promotion of Science]

593. Developmental competence in *Aspergillus nidulans*

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Developmental competence, the ability to respond to an induction cue, was first defined by Waddington in embryological studies (Waddington, 1940) and has since been examined in various systems including *Drosophila* eye development, *C. elegans* vulval development, marine invertebrate metamorphosis and recently, continuing work commenced in the 1950s, conidiation of *Penicillium* spp. Axelrod applied the concept to *Aspergillus nidulans* in the 1970s and found a minimum of 20 hours vegetative growth is required for a single colony to acquire competence, after which time conidiation can be synchronously induced by exposure to air and light (Axelrod, 1972). Competence in *A. nidulans* is an intrinsic property of development, influenced only by density.

Precocious (*pre*, renamed *prc*) mutants altered in their time of competence and temperature sensitive incompetent (*aco*) mutants were obtained in genetic screens by Axelrod, Champe and others (Axelrod et al., 1973; Butnick et al., 1984; Kurtz and Champe, 1979; Yager et al., 1982). These, newly isolated mutants and a number of candidate genes are being studied in order to understand the molecular basis underlying developmental competence. Phenotypic characterisation and mapping of these pleiotropic mutations has been performed. All precocious mutants identified so far are dominant.

Competence has also been investigated by comprehensive microarray expression profiling using wild-type, precocious and incompetent mutant strains. Transcriptional rewiring during competence acquisition is considerable, pointing to coordinated control of multiple pathways at and after competence. Axelrod, D. E., (1972) Kinetics of differentiation of conidiophores and conidia by colonies of *Aspergillus nidulans*. *J Gen Microbiol* 73: 181-184. Axelrod, D. E., Gealt, M. and Pastushok, M., (1973) Gene control of developmental competence in *Aspergillus nidulans*. *Dev Biol* 34: 9-15. Butnick, N. Z., Yager, L. N., Kurtz, M. B. and Champe, S. P., (1984) Genetic analysis of mutants of *Aspergillus nidulans* blocked at an early stage of sporulation. *J Bacteriol* 160: 541-545. Kurtz, M. B., and Champe, S. P., (1979) Genetic control of transport loss during development of *Aspergillus nidulans*. *Dev Biol* 70: 82-88. Waddington, C. H., (1940) Organisers and Genes. Cambridge University Press, UK. Yager, L. N., M. B. Kurtz and Champe, S. P., (1982) Temperature-shift analysis of conidial development in *Aspergillus nidulans*. *Dev Biol* 93: 92-103.

594. Withdrawn

595. Fungal genomics for biofuels

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The Joint Genome Institute (JGI) has sequenced and annotated over 25 fungal genomes with focus on organisms important for processes essential for biofuel production and ranging from biomass sustainable growth to degradation into simple sugars and then to biofuels. Our first genome analyses revealed secretome of the mycorrhyza *L. bicolor* essential for tree growth and divergence of mechanisms and enzymes involved in lignocellulose degradation by white rot *P.crysosporium*, brown rot *P.placenta*, and ascomycete *T.reesei*. Sequencing of *P. stipitis* strains with different capacity for ethanol production shed light on xylose fermentation. Ongoing comparative analysis of six Dothideomycete genomes is amed to find mechanisms of plant pathogenisity and host interactions.

Further sequencing and analysis of fungi will focus on phylogenetic breadth, resequencing selected targets, and probing ecological diversity with metagenomics. In addition to genome sequencing and in collaboration with communities we built transcriptomics and proteomics data to support genome annotation and analysis. Transition to new sequencing platforms increases the number of sequenced genomes and accuracy of their annotations supported by ESTs. JGI tools for automated annotation and web-based genome analysis help to keep up with the increasing genome flow. We built a Community Annotation program focused on training new users, achieving higher quality of annotations, and building active user communities for distributed genome analysis leading to practical applications for future biofuel development.

596. Morphogenetic mutants of *Phoma medicaginis*

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Phoma medicaginis is the causal agent of spring black stem and leaf spot on alfalfa (*Medicago sativa*) and related medics, including the model legume (*M. truncatula*). This necrotrophic pleosporalean fungus produces melanized pycnidia, which exude conidia, on the necrotized tissues of its host leaves and stems. Conidia are disseminated by rain splash and act as the principle inoculum. The genome of *P. medicaginis* has not been sequenced; therefore we used a forward genetics approach to study pycnidial development and pathogenicity. Using *Agrobacterium* mediated transformation, over one-thousand transformants have been generated. From these, six morphogenic mutants with single T-DNA tags and altered melanization patterns, or aberrant or absent pycnidia, were selected. An adaptor ligation PCR method was used to obtain genomic sequence flanking T-DNAs and identified three types of T- DNA insertions. In the first type, the T-DNA insertions disrupted the coding regions of a polyketide synthase (*pks*) and a poly-A polymerase (*cid13*). In the second type, the T-DNAs inserted in the promoter region or 3'-UTR of a cyclin-like protein and an unknown hypothetical protein conserved in eukaryotes, respectively. In the final type, the T-DNAs appeared to have integrated into intergenic regions. The transcription of tagged genes will be verified and open reading frames will be deleted by gene replacement to functionally regenerate mutant phenotypes. Efficient T-DNA tagging, a model host plant, and numerous genome sequences of related dothideomycete fungi make *P. medicaginis* a promising model plant pathogen.

597. Environmental amelioration of aberrant apical growth in the *Aspergillus fumigatus rasA* deletion mutant requires actin and Ca^{2+} / calmodulin mediated events.

Jarrod R. Fortwendel, Praveen R. Juvvadi, B. Zack Perfect, and William J. Steinbach

Deletion of *rasA* in the opportunistic pathogen *Aspergillus fumigatus* causes slow growth and the development of malformed hyphae, displaying aberrant apical growth processes. We have recently shown that culture of the *rasA* deletion mutant in RPMI media containing echinocandin (caspofungin, anidulofungin, or micafungin) at least partially ameliorates this apical growth defect. However, it remains unclear if the positive effect on growth direction is due to the specific inhibition of glucan synthesis or due to other non-specific effects of echinocandin treatment (i.e. slowed growth rate or other cell wall changes). Here we report that the aberrant apical growth phenotype is not affected by media type or culture temperature, but is partially corrected by the addition of sorbitol (1.2M) or polyethylene glycol [PEG] (15%) to the culture media. This implies that correction of the aberrant apical growth phenotype may not be directly due to the specific acitivity of glucan synthesis reduction caused by echinocandin treatment. In addition, correction of the aberrant apical growth pattern by culture in osmotically stabilized media (sorbitol or PEG) was inhibited by the addition of several agents to the growth medium, including: an actin depolymerizing agent, cytochalasin A, a calcium channel blocker, diltiazen, and a calmodulin inhibitor, trifluoperazine. This data suggests that the environmental remediation of apical growth in the *rasA* deletion mutant requires actin and calcium mediated events, including the Ca^{2+} / calmodulin signaling pathway. These data also resemble mutants of the MobB / CotA complex in *A. nidulans* and suggest that, as in yeast, the RasA pathway of *A. fumigatus* may share roles in polarized growth with the MobB / CotA pathway.

598. Transformation of *Trichoderma reesei* by electroporation of spores

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Most published electroporation methods for *T.reesei* use germinated cells pre- treated with cell wall-degrading enzymes. Here, we demonstrate that intact spores of *T.reesei* can be transformed by electroporation with reasonable efficiency leading to a high percentage of stable transformants. We also show that, in contrast to transformation of *T.reesei* spores by biolistic method, electroporation can be used for modification of chromosomal sequences via homologous recombination.

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599. Beta-glucosidases from an *Aspergillus* strain (Hj2) that expresses high amounts of lignocellulolytic enzymes: Screening and Gene Cloning.
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A broad range of fungi, isolated from various locations, were grown on lignocellulosic media and screened for lignocellulose degrading enzyme activities. Fungus Hj2, identified as a black *Aspergillus*, showed great enzyme potential for all enzyme activities tested. The fungal extract from solid state fermentation of Hj2 enhances the hydrolysis of pretreated lignocellulosic biomasses when combining it with the commercial enzyme preparation, Celluclast, from Novozymes A/S. Enzyme assays with 4- Nitrophenyl β-D-glucopyranoside as substrate indicate that the general beta- glucosidase activity of the fungus Hj2 is thermostable up to 60ºC. For cloning beta-glucosidase genes, conserved motif regions among known *Aspergilli* beta-glucosidase amino acid sequences are being used to design degenerated primers, which are applied for PCR screening of the fungus Hj2 genomic DNA. The full beta-glucosidase genes are then obtained through genome walking strategies. At present, one full length beta-glucosidase gene is cloned, and current work revolves around isolating more beta-glucosidase genes. Further work will focus on cloning and over-expression of the beta-glucosidase genes in a suited host. After fractionation of the fungal extract, the fraction which shows the greatest beta-glucosidase activity will be examined by LCMS to more specifically identify the enzymes that most efficiently hydrolyze the pretreated biomass.

600. Functional analysis of the two putative key players PRO11 and PHO1 reveals new insights into the protein-protein interaction network controlling fruiting-body development in *Sordaria macrospora*.
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The focus of our research work is on the interdependency of the components controlling fruiting-body development in the filamentous ascomycete *Sordaria macrospora*. Our actual study is based on the functional analysis of two putative key players in this complex differentiation process: *pro11* and *pho1*. The *pro11* gene encodes a multimodular WD40 repeat protein which shows significant sequence and functional homology to the mammalian protein striatin. Proteins belonging to the striatin family are thought to act as scaffolds linking signaling and eukaryotic endocytosis. It was found previously, that striatin forms a complex with the protein phocein. Little is known about phocein but in mammals it seems to be involved in vesicular trafficking processes.
By yeast two-hybrid analysis we showed that the *S. macrospora* homologs PRO11 and PHO1 are also able to interact with each other. To get deeper insight into the cellular function of both genes, we constructed knockout strains and deleted *pro11* and *pho1*. Both knockout strains exhibit a sterile phenotype which approves the importance of *pro11* and *pho1* during sexual reproduction. Here we show a detailed morphological characterization of the knockout strains. Complementation analysis of the knockout strains with truncated versions of PRO11 and PHO1 shed light on essential domains of both proteins. Furthermore, first results of Real Time PCR experiments and localization studies will be presented.

601. Unraveling hyphal differentiation in by genome-wide expression analysis of single hyphae
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Mycelial fungi use the hyphae growing at their apices to colonize a substrate. These hyphae secrete enzymes that convert complex polymers into small molecules that can be taken up by the fungus to serve as nutrients. Using GFP as a reporter it has been shown that exploring hyphae of *Aspergillus niger* are heterogenic with respect to enzyme secretion; some hyphae strongly express the glucoamylase gene *glaA*, while others express this gene lowly. This was a surprising finding considering the fact that all hyphae were exposed to the same nutritional conditions. Apparently, a vegetative mycelium is more complex than generally assumed. To establish whether hyphae differentiate into for instance secreting and non-secreting hyphae or whether several secretory types co-exist we want to perform a genome-wide expression analysis of hyphae highly and lowly expressing *glaA*. To this end, RNA will be extracted and amplified from both types of hyphae. We have set up protocols to collect the different hyphae by laser dissection using P.A.L.M and to isolate RNA from 1- 100 hyphae. QPCR has shown that we are able to extract RNA from few hyphae only. We have now started to amplify RNA to obtain sufficient amounts for hybridizations of whole genome arrays.
This research is supported by the Dutch Technology Foundation STW, applied science division of NWO and the Technology Program of the Ministry of Economic Affairs.

602. Novel tools for gene manipulations and a luciferase-based reporter system in *Neurospora crassa* reveal detailed real-time dynamics of *frq* /FRQ oscillations and uncovers new period mutants

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In every genetic system, the availability of tools for genetic recombineering and the availability of dependable and sensitive real time reporters are essential to progress. Furthermore, development of tools in one system often provides the springboard for dissemination of similar tools within the fungal genetics community. In *Neurospora crassa* the ability to perform very high (<98%) efficiency homologous gene replacements has fostered the development of a number of different knock-out, knock-in, and gene tagging strategies that have vastly expanded the repertoire of tools available. In addition, the recent development of luciferase as a real time reporter has greatly facilitated monitoring gene and protein expression and regulation. Application of these novel tools to the study of rhythms in *Neurospora* provides a revealing list of case studies. The *Neurospora* circadian oscillator is composed of a transcriptional translational negative feedback loop, where the phosphoprotein Frequency (FRQ) inhibits its own expression by affecting the activity of the white collar transcriptional complex (WCC), giving as a result robust oscillations in FRQ protein and message levels. These rhythms can be indirectly followed by the overt circadian regulation on spore formation (conidial banding) although technical limitations restrict the routine molecular analysis of *frq* message and protein levels to no more than 2-3 days. To overcome this and other limitations we have developed a fully-codon optimized luciferase reporter system for *Neurospora crassa*. Thus, by putting this real-time reporter under the control of the entire *frq* promoter, or discrete regions containing circadian elements, rhythms in *frq* transcription can be easily tracked for over a week. Moreover, by generating FRQ-LUC translational fusion strains, rhythms in FRQ protein can be followed in a semiquantitative manner. We have combined this bioluminescence-based system with a variety of tools for gene manipulation generated in association with the *Neurospora* Genome Project, such that different deletions strains can be easily analyzed for circadian molecular phenotypes. These facilitate reporter and regulatable promoter knock-ins, N- and C- terminal epitope tagging with a variety of tags, and strains designed to facilitate specific screens. Moreover, the circadian oscillator of strains with severe growth defects or no defined "banding" can now be revealed, an otherwise daunting task, when performed by classic western/northern approaches. In addition to genetic analyses, pharmacological perturbations can be easily performed, altogether revealing unexpected molecular details of the clockworks.

603. A simplified method for generating uracil auxotrophs in industrial fungal strains.

Shuang Deng, Kenneth S Bruno, David E Culley and Scott E Baker. Pacific Northwest National Laboratory

Filamentous fungi are capable of producing a range of valuable products. New strains and species that make these products are often not amenable to genetic modification using common resistance markers such as hygromycin. Consequently, the development of auxotrophic strains is important for generating gene deletions and other insertions. We have developed a straightforward method for generating pyrimidine auxotrophic mutants in fungi using homologous integration of a small PCR fragment. A 21 nucleotide sequence containing stop codons in each reading frame, was introduced into the native orotidine-5'-phosphate decarboxylase (*pyrG*) coding sequence using fusion PCR. Transformation of strains of interest to pyrimidine auxotrophy was achieved by homologous recombination of the mutant PCR product with the native *pyrG* gene. Pyrimidine auxotrophs were selected by plating on minimal medium containing uracil/uridine and 5-fluoroorotic acid. Transformants were confirmed by PCR and phenotype. The resulting *pyrG* mutant strains are easily complemented back to pyrG+ using the commonly available *pyrG* marker from *Aspergillus fumigatus* or *pyr-4* from *Neurospora*. Uridine auxotrophic mutants of production strains of *A. niger*, *A. oryzae* and *Trichoderma reesei* were successfully produced using this method. These *pyrG* mutants have proven useful in making gene knockouts and constructing gene expression systems.

604. A MAP kinase required for successive tip formation during Citric Acid Production in *Aspergillus niger*

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Routine growth of *Aspergillus niger* includes the formation of long branching hyphae that form a web of filaments forming a mycelium. During citric acid production, filament formation is suppressed and bulbous chains of cells aggregate into small pellets that are devoid of any extensive filamentation. The subsequent addition of manganese to a production culture results in the resumption of polar growth as well as a cessation of citric acid production. Previous observations indicate that these pellets contain multiple tips emanating from individual cells. In an effort to understand signaling mechanisms that control pellet formation, we deleted several MAP Kinsae genes in a citric acid production strain of *A. niger* (ATCC11414). Gene deletion of an orthologue of *Magnaporthe grisea* PMK1 (Xu and Hamer, 1996) resulted in a strain with reduced citric acid production. This mutant displays an atypical pellet morphology with extensive apolar growth and an inability to form large pellets. Addition of manganese restores filamentation, however, this MAP kinase mutant has fewer extending hyphae than the parental strain. This is an indication that the mutant is incapable of initiating multiple sites of polar growth under these conditions.

605. Peptide-assisted annotation of the *Melampsora larici-populina* genome

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Annotation of protein-coding genes is a key goal of genome sequencing projects. In spite of advances in gene finder programs, computational identification of complete gene models in eukaryote genomes remains a challenging task. Proteomic data and bioinformatics were used to refine the annotation of the poplar leaf rust fungus *M. larici-populina*. Total proteins were extracted from urediniospores, fractionated by 1D SDS-PAGE, trypsin-digested and analysed by mass spectrometry. A corpus of 876 000 tandem mass spectra was searched against two protein databases: a database containing the protein sequences from the ab initio predicted gene catalog, and a second protein database created by a six-frame translation of the genome. Identified peptides validated the current annotation of 1659 genes, which represent about 10% of the predicted genes in *M. larici-populina*. Peptides only found on the six-frame translation database suggested modification to the current annotation for 99 genes and identified 73 novel genes that were not part of the *M. larici-populina* protein-coding gene catalog. Our results highlight the benefit of integrating proteomic data to genome sequencing project.

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606. Multidrug efflux in ectomycorrhizal interaction

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Detoxification is necessary for every organism to prevent its cells from toxic compounds. Multidrug efflux pumps play an important role in this mechanism. In the last years, genes of the multidrug and toxic compound extrusion (MATE) family could be investigated and were identified as transporters for a wide range of substances including ethidiumbromide, antibiotics or Cd^{2+}. Although the function of some paralogs from bacteria, human and *A. thaliana* MATE genes were examined, only little is known about the role of MATE in fungi. For ERC1 from *S. cerevisiae*, the first and only investigated fungal MATE gene, the mediation of ethionine resistance was shown. The fungal MATE gene, *TvMATE1*, was found to be higher expressed in ectomycorrhizal interactions between *Tricholoma vaccinum* and its host spruce as compared to pure cultures of the fungus. The entire sequence could be isolated. The resulting protein contains two MatE superfamily-domains and shows a similarity of above 55% to other putative MATE genes of basidiomycetes. To understand the role of TvMATE1 in the mutualism and to investigate the substances that may be transported, two heterologous expression systems, the sensitive *E. coli* strain KAM3 and the yeast mutant of ERC1, are used. In analogy to bacteria which use the antiport to detoxify antibiotics through MATE, the ability for fungicide transport will be tested. In addition, heavy metals and compounds of the defense system of plants and soil microorganisms could be substrates for transport and will be investigated.

607. C*A*DRE, Aspergillus Genomes and The Aspergillus Cloud.

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The Central *Aspergillus* Data REpository (C*A*DRE). is a public resource for viewing annotated genes predicted by various *Aspergillus* sequencing projects. It currently houses seven genomes and, at present, is the only source of the most recent *Aspergillus nidulans* annotation arising from the Eurofungbase re-annotation project. In order to provide the most up-to-date and cohesive information, the Eurofungbase project not only reviewed annotation but also gene structures and assembly. 58 previously unassigned contigs were incorporated into the assembly and supercontig orientation was corrected. 2626 genes were manually reviewed and, as a result the percentage of genes with informative names rose from 3% to 19%. After transfer of information by orthology, this rose to 58%. C*A*DRE (which serves approx. 20,000 pages per month) has also recently merged with another significant Aspergillus resource, the *Aspergillus* Website (which serves approx. 960,000 pages per month), to form 'Aspergillus Genomes'. The *Aspergillus* Website has primarily served the medical community, providing information about Aspergillus and associated diseases to medics, patients and scientists. Thus, by merging these databases, genomes benefit from extensive cross- linking with medical information to create a unique resource, spanning genomics and clinical aspects of the genus. In addition, to enable the exploration of inherent links between data presented within this website, we have introduced the Aspergillus Cloud. C*A*DRE and Aspergillus Genomes are accessible from http://www.cadre- genomes.org.uk and http://www.aspergillus-genomes.org.uk, respectively.

608. Over-expression and purification of *Trichoderma reesei* glycosyl hydrolases in *Pichia* and in *T. reesei*.

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The high cost of degradative enzymes is a limiting factor in the economical conversion of lignocellulose to fermentable sugars. All current biomass-degrading commercial enzyme mixtures are derived from a handful of Ascomyceteous fungi, especially *Trichoderma reesei* and *Aspergillus niger*, but the enzymatic activities and relative proportions of current enzyme mixtures are poorly defined. The goal of this project is to produce a defined enzyme mixture that is optimized for pretreated corn stover. On the basis of abundance and predicted importance, twenty enzymes from a proteomics analysis of *T. reesei* were selected to be expressed either in a heterologous host (*Pichia pastoris*) or in *T. reesei* itself. Several expression vectors for *T. reesei* have been constructed that use either a strong constitutive promoter or a strong cellulose-inducible promoter from *T. reesei*. The long-term goal is to develop an optimized set of enzymes that has higher specific activity on real lignocellulosic materials than current industrial enzymes. These experiments will also help us identify the key enzymes that should be targets of improvement and further research.

609. Interactionpartners of *Aspergillus nidulans* phytochrome FphA

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Perception of light is important in all kingdoms to adapt to environmental cues and is mediated by photoreceptor molecules. In *A. nidulans* a multi-component light receptor complex has been proposed, suggesting cross-talk between different light quality signaling pathways. The light-regulator complex triggers development and secondary metabolism. The protein complex consists of the phytochrome FphA, the regulator protein VeA, and the white collar homologues LreA and LreB. The phytochrome FphA reassembles of a light sensory domain and a signal output domain, consisting of a histidine kinase and a response regulator domain. Previously it was shown that the phytochrome FphA directly interacts with the WC-2 homologue, LreB and the regulator VeA. We mapped the interaction of FphA with LreB to the histidine kinase and the response regulator domain at the C-terminus. In comparison, VeA interacted with FphA only at the histidine kinase domain. We present evidence that VeA occurs as a phosphorylated and a non-phosphorylated form in the cell and we propose a model for the transmission of the light signal to downstream regulators.

610. Construction of quintuple protease gene disruptant for heterologous protein production in *Aspergillus oryzae*.

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Aspergillus oryzae has received attention as a host for heterologous protein production. However, A. oryzae has 134 protease genes, which is recognized to be one of the major reasons for the proteolytic degradation of heterologously produced proteins. We previously reported that double disruption of the protease genes (*tppA*, and *pepE*) improved heterologous protein (human lysozyme) production by *A. oryzae*. In this study, we performed successive round of five protease genes (*tppA*, *pepE*, *nptB*, *dppIV*, and *dppV*) disruption in *A. oryzae* by *pyrG* marker recycling with highly efficient gene-targeting background (*f¢ligD*). The multiple disruption of protease genes were confirmed by Southern blot analysis. Furthermore, the quintuple protease gene disruptants showed the maximum production level of bovine chymosin (CHY) that was 34% higher than those of the double protease gene disruptant (*f¢tppA f¢pepE*). Consequently, we successfully constructed a multiple protease gene disruptant bearing enhanced levels of CHY productivity. For construction of *A. oryzae* with more improved heterologous protein productivity, additional two rounds of protease gene (*alpA*, and *pepA*) disruption from the quintuple protease gene disruptant (*f¢tppA f¢pepE f¢nptB f¢dppIV f¢dppV*) is being performed.

611. Molecular epidemiology of *candida* spp. In gynecologic patients of Ensenada, Baja California, Mexico.

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In the last years, *Candida* spp. have emerged as major opportunistic pathogen, s. *C. albicans* is the most frequent species, nevertheless species such as *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* have increasingly been isolated from people with wide distribution. One of the most common infections by *Candida* is in gynecologic patients Our goal was to study the diversity and distribution of *Candida spp.* among gynecologic patients from the Ensenada General Hospital, 140 women were included, the vaginal swabs were first inoculated in YPD and the recovered colonies were analyzed using a PCR-based method with primers ITS1 and ITS2 and subsequently by RFLP using MspI as a restriction enzyme. The 42.8% of the women included in this study were positive for *Candida* (PCR). By comparing the PCR-RFLP of the vaginal isolates, all could be identified to the species level, except in two cases, one that produced a no recognizable pattern and the other that was not Candida. The latter was identified as *Geotrichum candidum*. *C. albicans* was the predominant species (55.0 %), followed by *C. glabrata* (11.6%), *C. guilliermondii* (11.6%) and *C. tropicalis* (5.0 %). It was possible to differentiate two species in 15 % of the vaginal samples. As in many studies, *C. albicans* was present in more than the half of the positive samples, however it is very important to identify the species throughout simple molecular based methods that make easy to know the responsible of the infection in order to recommend an adequate antifungal treatment.

612. Antifungal Evaluation of Novel Benzotriazole and Ebselen Derivatives against several fungal strains.

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In an effort to develop potent antifungal agents a series of fluconazole analogs, such as substituted benzotriazoles, was prepared and evaluated for antifungal activity against *Candida* spp., and *Aspergillus* spp. *in vitro*. Several of these analogs were found to be superior to fluconazole. Seven of these compounds were tested in concentrations ranging from 1 to 100 microM for their ability to inhibit growth of *Aspergillus nidulans* R153 in liquid rich medium. Of the seven only one inhibited growth completely at 30 and 100 microM. The others were less effective. A detailed synthesis and structure activity relationship (SAR) of these analogs will be discussed. Ebselen had previously been shown to effectively inhibit growth of *S. cerevisiae* in a dose-dependent manner. (J. Biochem Mol Toxicol. 2007 21:252-264). It was tested in doses ranging from 1 to 100 microM against two strains of *A. nidulans* on solid and in liquid rich media. It had no effect on growth on solid medium but effectively inhibited both strains in liquid medium at a concentration of 100 microM. Supported in part by I.M.S.D. grant to St. John's University and NIGMS R15GM077345-01A1.

613. A lectin-mediated defense of fungi against predators and parasites

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The galectins CGL1 and CGL2 as well as the homologous lectin CGL3 of the homobasidiomycete *Coprinopsis cinerea* are strongly induced during sexual development and highly enriched in the fruiting body. Neither ectopic expression during vegetative growth nor silencing of the respective genes had any obvious effect on fruiting, suggesting that these lectins are not involved in mushroom development. Instead we found a pronounced, carbohydrate-binding-dependent toxicity of the galectin CGL2 towards the nematode *Caenorhabditis elegans*, two different insect larvae (*Aedes aegypti, Drosophila melanogaster*) as well as two different types of amoebae (*Acanthamoeba castellanii, Dictyostelium discoideum*). We tested a panel of additional characterized fungal lectins for toxicity towards the above mentioned organisms by feeding them with E. coli cells expressing the respective proteins. In this type of assay, most of these lectins were toxic and some were very selective with regard to the target organisms. Glycan-array analysis, in combination with genetic studies in *C. elegans*, allowed us to determine the target glycans of toxic fungal lectins and to start to unravel the mechanism of lectin-mediated nematotoxicity. Furthermore, we set up laboratory cultures of the fungivore nematode *Aphelenchus avenae* in order to evaluate the physiological significance of nematotoxic lectins for the fungus in co-culture with this predator. Our data suggest that the presence of the predator is able to induce the expression of CGL2 in the vegetative mycelium of both, monokaryotic and dikaryotic *C.cinerea* strains. We propose that homobasidiomycetes and possibly other multicellular fungi have an inducible innate immune system that includes a lectin-mediated defense against predators and parasites.

614. Electrophoretic and cytological karyotyping of the chestnut blight fungus, *Cryphonectria parasitica*.

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We analyzed karyotype of the chestnut blight fungus, *C. parasitica*, by combining cytology and pulsed-field gel electrophoresis (PFGE). In cytological karyotyping, fluorescence microscopy on the mitotic metaphase specimens prepared by the germ tube burst method showed *n*=9 for both standard wild type strain EP155 and virus- infected field strain GH2. Each chromosome complement in the two strains was characterized by relative size, heterochromatic segment and constriction, and the karyotypes incorporating such characteristics were different between EP155 and GH2. We found that differential staining using actinomycin D as a counter stain prior to DAPI- staining was effective to highlight constitutive heterochromatin. PFGE for 9 strains including 6 field stains and 3 transformants resolved 5 to 6 bands with sizes ranging from 3.3 Mbp to 9.7 Mpb. Except for GH2, banding profiles were identical among the strains, irrespective of their geographic origin and harboring or non-harboring of viruses. Southern blot analysis with single copy genes as probes revealed that the difference of GH2 from the others was partly due to chromosome translocation that was also manifested in the cytological karyotypes. Telomere fingerprints for EP155 and GH2, on the other hand, showed relatively high level of similarities suggesting conservation of subtelomeric regions. Integration of the data from cytology and PFGE revealed that the genome size for EP155 and GH2 was estimated to be *ca.* 50 Mbp. This is the first report of a cytological karyotype in the order Diaporthales.

615. Biotechnological application of xylanases and ligninases produced by *Aspergilli* cultivated on wheat bran, on cellulose pulp biobleaching

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Aspergillus fumigatus and *Aspergillus niveus* were isolated from Brazilian soil using a Bioprospection program. These fungi were excellent xylanase producers and were cultivated at 40°C, static conditions, in Vogel or Czapeck minimum liquid medium supplemented with 1% xylan, during 96 or 120 hours for *A. fumigatus* and *A. niveus*, respectively. The xylanase production was either high in agroindustrial residues as rice flakes, wheat bran, crushed corncob, powdered corncob and rice straw - *A. fumigatus*; wheat bran and powdered corncob - *A. niveus*. For ligninases (laccase, Mn-P and Li-P) the culture conditions of *A. niveus* were optimized in SSF using wheat bran as carbon source, during 14, 21 and 35 days, respectively. In cellulose pulp biobleaching the results were promissory: *A. niveus* xylanase reduced 4.6 points kappa number, increased whiteness 3.4 points, and maintained the viscosity. Xylanase from *A. fumigatus* reduced 0.9 points kappa, increased whiteness 2 points, and reduced viscosity 9.2%. With the mix of xylanases/ligninases from *A. niveus* there was a reduction of 6.5 points in kappa, an increase of 17.2 points in whiteness and viscosity was reduced only 1 point. The efficiency of these treatments was confirmed using Scanning Electronic Microscopy.

616. Distinct roles of three G protein alpha-subunits and an adenylyl cyclase in fruiting body development of the homothallic fungus *Sordaria macrospora*.

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The self-fertile ascomycete *Sordaria macrospora* carries genes (*gsa1-3*) encoding three different alpha-subunits of heterotrimeric G proteins. We generated knockout strains for all three genes as well as all combinations of double mutants. To test whether the pheromone receptors PRE1 and PRE2 mediate signaling via distinct Galpha-subunits, two recently generated delta-pre strains were crossed with all delta-gsa strains. The analyses of the mutants revealed that compared to GSA2, GSA1 is a more predominant regulator of a signal transduction cascade downstream of the pheromone receptors and that GSA3 is involved in another signaling pathway that also contributes to fruiting body development and fertility. We further isolated the gene encoding adenylyl cyclase (AC)(*sac1*) for construction of a knockout strain. Analyses of the three delta-gsa delta-sac1 double mutants and one delta-gsa2 delta-gsa3 delta-sac1 triple mutant indicate that SAC1 acts downstream of GSA3, parallel to a GSA1–GSA2-mediated signaling pathway. From the sum of all our data,we propose a model for G protein mediated signaling in sexual development of *Sordaria macrospora*.

617. *Neurospora crassa* HSF2 is required for asexual development.

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We have identified two genes in the *Neurospora* genome encoding proteins with similar domains to classic heat shock factors. We have shown that *hsf1* is an essential gene and that *hsf2* is required for asexual spore development. Overall there was little difference in the expression levels of *heat shock protein*(*hsp*) genes *hsp90, hsp70* and a *hsp90*-associated transcript in control and heat shocked cultures of wild-type and the $hsf2^{KO}$, indicating that HSF2 is not absolutely required for this response. Furthermore, knockout strains of *hsp90a, hsp30, hsp78*, and *hsp101* have no obvious defect in conidiation. To place HSF2 in the known conidiation pathway we investigated the expression of *hsf1, hsf2*, and conidiation- associated genes *fluffy, con-10, rco-1* and *eas* in wild-type, *hsf2, acon- 2* and *fluffy* mutants at different times through development. We found that *hsf2* transcript levels were not significantly different in any of the strains examined. Moreover, wild-type levels of *fluffy* and *con-10* were found in the mutant suggesting that HSF2 acts either post-transcriptionally to affect the activity of these gene products or in a parallel pathway. While this finding mirrors the situation in animals and plants, where members of the family of heat shock factor proteins are known to function in an increasing number of development pathways, this is the first case where a developmental role has been shown for a heat shock factor in a filamentous fungus.

618. Construction of vectors for targeted gene replacement in fungi by USER Friendly cloning

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Functional genetics in filamentous fungi have always been dependent on the isolation or construction of mutant strains. The genome sequencing of over 100 fungi genomes has increased the need for faster and efficient methods to construct targeted replacement and overexpression mutants. To accommodate this we have developed a new vector system that allows single-step construction of vectors for targeted genome modification, thereby cutting vector construction time from ten to only three days and removing half of the required work load. The vector system is dependent on the Uracil-Specific Excision Reagent cloning technology (USER Friendly), which in its commercial version offers high efficient directional cloning of a single PCR amplicon [1]. However, our research shows that USER friendly cloning also can be used for the simultaneous directional cloning of several PCR amplicons and vector fragments, with a cloning efficiency of 85 %, thus allowing single-step construction of replacement vectors. The new vector system includes vectors for: gene replacement, promoter exchange, ectopic overexpression and general purpose cloning [2]. The system has been utilized to analyse the PKS3 gene cluster in *Fusarium graminearum*.

[1] New England Biolabs (2008), "USER Friendly Cloning Manual" [2] Frandsen et al. (2008), BMC Molecular Biology, 9:70

619. The in- and outside of *Epichloë* festucae: genome, genes, and gene expression

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Epichloë festucae, is a model endophyte that 1) switches between mutualistic and antagonistic states, 2) is seed transmissible, 3) is rich in bioprotective alkaloids, and 4) has a manageable genome size of an estimated 29 ± 3.5 Mb. This fungus grows systemically and intercellularly throughout the life of its host plant. On each reproductive tiller the fungus either infects benignly and transmits clonally in seeds, or produces its sexual state (stroma) and chokes inflorescence development. Here, we present the annotated genome of *E. festucae* consisting of 1497 supercontigs, and covering a net length of 27.4 Mb. We constructed 11,559 mRNA models by merging 8,557 *E. festucae* unigenes with gene predictions, and annotated them using BLAST, InterPro, Blast2GO, SignalP, TargetP, and TMHMM. The genome is accessible through GBrowse at http://csbio-a.csr.uky.edu/cgi/gbrowse/efgenome/. Analysis of differential gene expression between benignly-infected inflorescences and stromata revealed changes in fungal cell wall components, carbohydrate utilization, as well as membrane lipid, nitrogen, amino acid, and energy metabolism. Among the most highly upregulated in inflorescences were secondary metabolism genes and secreted protein genes. Potential adaptation mechanisms to different environments during the ectophytic and endophytic growth stages of *E. festucae* are discussed.

620. Protoplast-mediated transformation of a filamentous fungus *Cladosporium phlei* and evidence for the high frequency of tandem repeats of integrative transforming vector.

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Hypomycetous fungus *Cladosporium phlei* (C. T. Gregory) de Vries, known as a causal agent of leaf spot disease of timothy (*Phleum pratense*), is characterized by the production of deep red pigmented mycelia due to the presence of fungal pigment, phleichrome. Phleichrome belonging to a group of fungal perylenequinone has gained a great attention because it can be further converted to a photodynamic therapeutic (PDT) agent such as hypocrellin and its derivatives. In order for the genetic manipulation of this fungus, protoplast-mediated transformation of *C. phlei* has been developed and the resulting transformants were characterized in this study. Hygromycin B resistance was applied as a dominant selection marker due to the sensitivity of *C. phlei* to this antibiotic. The transformation efficiency ranged from approximately 20-100 transformants per experiment. Southern blot analysis of stable transformants revealed that transformation occurred via stable integration of the vector DNA into fungal chromosome. PCR analysis and plasmid rescuing of randomly selected transformants suggested that an integration of tandem repeated-copies of vector DNA was common. In addition, more than two integrations of the transforming vector at different sites were also observed. The establishment of transformation method of *C. phlei* has great utility for the various molecular breeding of this strain and represents an initial step in the molecular analysis of pigment production in this fungus

621. Differential expression of invertase by *Aspergillus niger* immobilized into polyurethane foam.

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Solid State Fermentation (SSF) is an alternative culture system for protein production by filamentous fungi. It has been reported that SSF allows higher protein production, shorter fermentation times and the induction of alternative proteins forms. Despite all those advantages, molecular understanding of SSF is yet in the primary stages, among other reasons, because agroindustrial by-products are used as support, making SSF cultures too complex to be analyzed. Immobilization of fungi in polyurethane foam (PUF) as an inert support has allowed our working group to obtain higher invertase production levels. We have also achieved shorter fermentation times and proteins free from contaminating materials derived from the breakdown of the solid support, since PUF is not metabolized by *A. niger*. Analysis of the invertase produced by SSF in PUF, using isoelectric focusing showed only one band with a pI of 4.7. Meanwhile, in shaken flask, two invertase isoforms were produced showing pI values of 3.6 and 4.4. The fact that the sequence of the genome of *A. niger* is complete and contains only one extracellular and two intracellular genes will help to study the transcription and transduction patterns of such enzyme system as a model for the comparison of SSF and SmF at molecular level. This Project is financed by the Public education Secretary of México by PROMEP program.

622. Investigating the roles of the *CDC7* and *DBF4* genes during meiosis in *Saccharomyces cerevisiae*.
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We have been examining the interactions of the *CDC7* and *DBF4* genes during yeast meiosis. We have found that high-copy *DBF4* suppresses the sporulation defect of the *cdc7-1* mutation, suggesting that the two gene products interact in meiosis as they do during mitotic growth. We recently performed co- immunoprecipitation experiments to identify other proteins with which the Cdc7 protein interacts during meiosis. In addition, we are screening a high-copy library for suppressors of other alleles of the *CDC7* gene. Finally, we have examined suppression of the *cdc7* and *dbf4* mutations by *bob1*, an allele of the DNA replication gene *MCM5/CDC46*. Although *bob1* completely suppresses the mitotic defect of these mutations, *bob1* only partially suppresses them in meiosis, allowing increased sporulation of the *cdc7* and *dbf4* mutants in the form of dyads. These data indicate that the Cdc7 and Dbf4 proteins work together to perform their function during meiosis. In addition, the partial suppression of the *cdc7* and *dbf4* mutations by *bob1* is consistent with these two genes having a different role in meiosis compared to mitotic growth.

623. Live-cell imaging reveals that arbuscular mycorrhizal fungi inherit and require hundreds of nuclei to survive.
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Biological inheritance involves the passing of traits from parents to offspring, with the genetic variation exhibited by individuals contributing to the evolution of the species. However, species evolution is less well understood for organisms undergoing asexual reproduction. One such group is represented by the arbuscular mycorrhizal (AM) fungi, ancient plant root symbionts, ubiquitous in most ecosystems that reproduce asexually via multinucleate spores. The mechanisms by which these spores are formed and the origin of the nuclear population provide an ideal model system to examine the genetic basis of evolution in coenocytic organisms. We use live real time cellular imaging to show the transmission of hundreds of nuclei into developing AM fungal spores. We find a significant positive relationship between spore size and nuclear content per spore for four AM fungal taxa as well as a surprising heterogeneity in nuclear content among spores. More importantly, we find that spores containing less than one hundred nuclei do not germinate. In contrast to all other known organisms, in which all somatic nuclei derive from a single progenitor nucleus, we discovered that AM fungi have a non-conventional system of heredity that has fascinating implications for the diversity of modes of evolution in eukaryotes.

624. Molecular diagnosis of *coccidioides* spp. In environmental samples: exploring the fungal ecological niche in North America deserts.
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Coccidioidomycosis is an emerging human fungal infection endemic of North American deserts. In most cases, the primary mycosis is self-limiting. However, a considerable number of patients develop a disseminated form of the disease with high morbidity and mortality. Despite the high prevalence rates of the disease in arid lands, reports of positive detection of the causal agent, *Coccidioides* spp., from environmental samples have been traditionally scarce. To resolve this paradox, we proceeded by first evaluating the predicted ecological niche of this fungus, and subsequently used improved available molecular tools to identify *Coccidioides* spp. in samples collected from areas of Baja California, Mexico previously predicted as endemic "hotspots". Using available epidemiological information on Coccidioidomycosis and data on Ecological niche modeling generated with *Genetic Algorithm for Rule Set Production* (GARP) and Geographical information Systems (Baptista-Rosas et al. 2007), we designed sampling polygons in Valle de las Palmas and San José de la Zorra. By nested PCR we explored the presence of *Coccidioides* spp. in heteromyids´ burrows, latrines and paths. The 170bp amplicons obtained were sequenced and compared to the Genebak database BLAST, showing identity with the ITS2 region of *C. posadasii* (E value 5e-83). Overall, 58% of the samples were positive (23 of 40 samples). Valle de las Palmas, a well known highly endemic area near Tecate in Northern Baja California, was the area with the highest detection rates (67%, 20 of 30 samples).

625. Analysis of 14 *Coccidioides spp.* genome sequences highlights incomplete speciation and natural selection.
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We have fully sequenced the genomes of 4 *C. immitis* isolates and 9 *C. posadasii* isolates, allowing us to explore regional variation in patterns of intraspecific diversity and interspecific divergence. These genome sequences, in combination with the *C. posadasii* C735 genome previously sequenced by JCVI, offer an excellent resource for improving our understanding of these dimorphic fungal pathogens, which are the etiological agent of coccidioidomycosis (Valley Fever). Through analysis of all 14 genomes we find that although *C. immitis* and *C. posadasii* nominally diverged at least 5 million years ago, extensive regions of their genomes exhibit evidence of recent gene flow even while the majority of the genome exhibits perfect genetic isolation. We explore the relative importance of neutral factors vs. natural selection in creating these patterns of heterogeneous divergence. Delineation of the genomic regions where gene flow occurs between these species may be useful in guiding the development of vaccines or other therapeutics that will be equally effective for both taxa. More broadly, we explore the signal of natural selection across all genes in both species, and identify signals of balancing selection in secreted and membrane or cell wall associated proteins. These selection signals may indicate that immune-mediated selection pressure from mammalian hosts is an important driver of *Coccidioides* evolution, and help to clarify the relative importance of the saprophytic vs. parasitic phases of the *Coccidioides* life cycle.

626. Development of a recombinant Trichoderma strain for improved hydrolysis of pretreated corn stover

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A majority of currently available commercial enzyme products for biomass hydrolysis are produced by the saprophytic mesophilic fungus *Trichoderma reesei*. *Trichoderma* produces two cellobiohydrolases (CBHI and CBHII), five endoglucanases (EGs), and two beta-glucosidases (BGs). Although this mix is relatively efficient at cellulose degradation and large quantities of these proteins are secreted from the fungus, improvements in the total enzyme specific activity and secretion yield may improve this product. A primary factor in the high cost of enzymes for biomass hydrolysis is the amount of enzyme that must be applied for efficient cellulose conversion to glucose. Compared with starch hydrolysis, 15-100 fold more enzyme is required to produce an equivalent amount of ethanol, depending on specific process conditions. It is well known that efficient cellulose hydrolysis requires a complex, interacting mix of cellulose degrading proteins. To significantly reduce the enzyme loading required, one may replace *Trichoderma* components with more efficient candidates, or augment the enzyme system with additional components to improve the overall enzyme performance. In this study, identification of new genes that improve specific performance in hydrolysis of pretreated corn stover, and their expression in *Trichoderma* will be discussed.

627. Development of high-throughput methods for heterologous expression in fungi.

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The rapid increase in the number of sequenced organisms has resulted in an explosion in the number of genes that are desired to heterologously express. However, heterologous expression requires the creation of DNA constructs which is the bottleneck in the construction of strains of interest in most projects. One promising technique to overcome this is uracil-excision based cloning which was first described in the early 1990s. This technology has been available as a commercial kit for several years (USER™) however, the technology has remained largely unused. We have made several essential modifications to this technology which now allows simultaneous fusion and cloning of multiple PCR products independently of restriction sites. We will present the collection of plasmids we have constructed, which has made the generation of constructs for heterologous expression in fungi simple and fast.

628. Computational biodiversity analysis and identification/characterization of a new laccase from the cellulolytic fungi *Trichoderma reesei*

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The growing number of fungal genomes sequenced offers an opportunity to evaluate and compare the potential of fungi for the degradation of lignin and related aromatic compounds. Indeed, the breakdown of lignin by fungi is a key step during carbon recycling in terrestrial ecosystems and this process is of great interest for green and white biotechnological applications. Given the importance of these enzymatic processes, the enzymes potentially involved in lignin catabolism were classified into sequence-based families and integrated in a newly developed database, designated Fungal Oxidative Lignin enzymes (Levasseur et al. 2008, http://foly.esil.univ-mrs.fr). This database efforts aims at providing the means to get new insights for the understanding and biotechnological exploitation of the lignin degradation.
Complete functional annotations of the potential ligninolytic system of Basidiomycota and Ascomycota were carried out, lending first comparative insight into the diversity of fungal lignin degradation. Among fungal genomes screened, genome of the cellulolytic *Trichoderma reesei* QM6a was annotated. To date, no laccase activity has been described in *T. reesei* and interestingly, candidate enzymes related to laccases were inferred. Laccases are blue copper oxidases catalysing the one-electron oxidation of phenolics, aromatic amines and other electron-rich substrates with a concomitant reduction of O_2 to H_2O; thus, laccases have been demonstrated to be useful tools in industrial processes in the pulp and paper or in bioenergy sectors.
After cloning and homologous overexpression of the candidate laccase gene identified by genome mining, the corresponding recombinant protein was purified to homogeneity and fully biochemically characterized. Evolutionary studies were performed in order to unravel the history of this new laccase and to provide its phylogenetic relationships in the multicopper oxidases family.

629. Analysis of repeat regions in the *Pyrenophora tritici-repentis* genome reveals gene expansion due to transposition.

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The Dothideomycetes represent a class of fungi that contain a large number of plant pathogens. One of the members of this class, *Pyrenophora tritici-repentis* (Ptr), is the causal agent of tan spot of wheat. The disease tan spot is influenced by the production of host-selective toxins (HSTs). Races of Ptr are distinguished by the production of one or a combination of HSTs. In highly virulent race 5 isolates of Ptr there are multiple identical copies of the HST gene *ToxB*. Isolates that contain fewer copies of the gene are less virulent. Therefore, gene expansion could contribute to increased virulence. The genome of a race 1 isolate of Ptr has recently been sequenced and approximately 16% of its sequence is repetitive. A large number of these repeats are 95-100% similar. Some protein families found within these repeats were identified as transposons. However, there are non-transposon protein families also found in repeated regions. For example, there is an expansion in the Histone H3 family where there are 25 copies of a Histone H3-like gene that are 99-100% identical. Interestingly, these genes are located in close proximity to two different hAT-containing DNA transposons. All 25 transposases associated with the Histone H3-like genes share highly conserved N-terminal sequences. However, the C-terminal sequences separated these proteins into two groups containing 10 and 15 proteins respectively, and within each group the C-terminus is highly conserved. These data suggest a mechanism for gene family expansion and sequence conservation in Ptr.

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630. Profiling a killer: Effects of PtrToxB on gene expression in wheat.

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The fungal wheat pathogen *Pyrenophora tritici-repentis* (PTR) produces several host-selective toxins (HSTs). Two proteinaceous HSTs produced by PTR, PtrToxA (ToxA) and PtrToxB (ToxB) are characterized and both evoke a susceptibility response in the host. When infiltrated into leaves of the sensitive cultivar Katepwa ToxA induces necrosis and ToxB induces chlorosis. Understanding the events triggered in the host by these HSTs will provide valuable insight into the nature of host disease susceptibility. We previously analyzed differential gene expression in wheat triggered by ToxA. Major transcriptional changes were revealed, including upregulation of pathogenesis-related, oxidative stress-related and other genes, that are similar to those associated with a defense response. To evaluate whether there are consistent host responses triggered by different toxins we analyzed gene expression changes induced by ToxB. Here we present analysis of gene expression in ToxB- and mock-treated leaves of a ToxB-sensitive cultivar over time. We used GeneChip Wheat Genome Arrays from Affymetrix. Normalization and filtering of the data was done with Bioconductor software packages and relational database queries. Differentially expressed genes were identified between each treatment at each time point with four different statistical methods. For gene identification a new annotation for the GeneChip Wheat Genome Array was generated.

631. A HacA-dependent transcriptional switch releases *hacA* mRNA from a translational block upon ER stress

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Activation of the unfolded protein response (UPR) in eukaryotes involves splicing of an unconventional intron from the mRNA encoding the transcriptional activator of the pathway. In *Saccharomyces cerevisiae* a 252 nt unconventional intron is spliced out of the transcript of HAC1, changing the 3'end of the HAC1 ORF and relieving the transcript from a translational block in a single step. The translational block is caused by base-pairing of part of the unconventional intron with the 5'UTR. In *Aspergillus niger* and other Aspergilli the unconventional intron in hacA mRNA is only 20 nt long. Since this intron is part of a stable stem- loop structure, base-pairing with the 5'UTR, in contrast to the yeast HAC1 case is not possible. However, analysis of the hacA mRNA revealed a GC rich inverted repeat (18 base pairings). Upon activation of the UPR, the 5'UTR of the hacA mRNA is truncated by 230 nt, removing the left part of this inverted repeat. This implies a similar release of a translational block as in the case of the *S. cerevisiae* HAC1, but in two steps. The mechanism behind the 5' truncation, which does not take place in either yeast HAC1 or mammalian xbp1 mRNA, has been unknown hitherto. Here we show that during secretion stress in *A. niger*, hacA transcription starts from a new start site closer to the ATG, relieving the transcript from translational attenuation. This transcriptional switch is mediated by HacA itself and the HacA binding site UPRE2 in the hacA promoter.

632. Studying the non receptor GEF RIC8 using Inhibitors and Suppressors in *Neurospora crassa*.

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RIC8 is a recently discovered regulator of G protein signaling that has been indentified in most eukaryotes including humans. It is essential for asymmetrical growth in embryogenesis as well as synaptic signaling. It is known to be a Guanine Nucleotide Exchange Factor for the Galpha's, but is neither a receptor nor membrane bound making it an interesting exception to canonical G protein signaling. To further elucidate its function, I am using the model organism *N. crassa*. *N. crassa* is an ideal system for these studies as *ric8* is not present in *S. cerviasae* or plants, and *N. crassa* grows via tip extension in vegetative culture providing an accessible surrogate for studying asymmetrical cell division. I am currently studying *ric8* in *N. crassa* using both suppressor analysis and chemical screening. The deletion of *ric8* induces severe pleiotropic effects and a nearly lethal growth phenotype. Through random mutagenesis I have generated suppressor mutants which recover part of the wild type phenotype in the *ric8* deletion background. The characterization of these mutants using SNP-CAPS should identify previously unknown players in same pathway as *ric8* and help to illuminate its exact signaling roles. In parallel, I am conducting a chemical screen for inhibitors and enhancers of the interaction between *ric8* and its Galpha's. Using compounds that specifically alter the *ric8*/ Galpha interaction *in vivo*, I intend to tease apart the effects of *ric8*'s interaction with G protein signaling and effects independent of G protein signaling.

633. High-throughput analysis of serine-threonine protein kinase genes in *Neurospora crassa*

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Serine-threonine (S/T) protein kinases are responsible for 99% of cellular phosphorylation events. Protein phosphorylation by S/T kinases is essential for signal transduction and many critical cellular processes. We annotated a total of 90 S/T kinase genes in the *Neurospora* genome, of which only 17 have been functionally characterized. Using a high throughput method viable gene replacement mutants were obtained for 80 S/T kinase genes. Phenotypic analysis of the these strains revealed more than 50% (41 out of 80) had defects in vegetative growth, or asexual or sexual development. A majority of the mutants having phenotypes (71%; 29 out of 41) exhibited defects or abnormalities in more than one function, suggesting that most S/T kinases regulate multiple functions. Compared to the large group of 103 transcription factor genes studied previously, more kinase genes regulate sexual development (~50% of kinases and ~18% of transcription factors, including ascospore lethal genes). To examine the roles of kinases in stress regulation, we analyzed the sensitivity of knockout mutants to oxidative stress generated by menadione and assayed phosphorylation of the three *Neurospora* MAPKs in several kinase mutants under hyperosmotic stress. Our current results will be presented.

634. A role for *MID1* and *CCH1* in ascospore discharge in *Gibberella zeae*.

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The majority of ascomycetes forcibly discharge their ascospores, and yet the mechanism of discharge is not well understood. We are studying two calcium channels, *MID1* and *CCH*1, which are involved in ascospore discharge in the filamentous fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). In G. zeae, delta *cch1* mutants were observed to have slower vegetative growth rates, abnormal sexual and ascospore development, and lacked ascospore discharge and cirrhus production as compared to wild type. The delta *mid1* mutants grew slightly slower, discharged ascospores at a slightly higher level, developed abnormal ascospores with much greater frequency than the delta *cch1* mutants. Complementation of the delta *mid1* mutants with wild type *MID1* sequence restored wild type characteristics. Delta *mid1*, delta *cch1* double mutants were also characterized and compared to the characteristics of the single mutants. Complete functional analysis of both genes and their effects on ascus function will be presented.

635. RIC8 regulates polar growth in *Neurospora crassa*.

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Heterotrimeric G protein signaling pathways are important for eukaryotic cells to respond to environmental stimuli, and consist of a GTP hydrolyzing G alpha protein and a G beta/gamma dimer. Traditionally, heterotrimeric G proteins are coupled to G protein coupled receptors (GPCRs), which serve as guanine nucleotide exchange factors (GEFs) for G alpha subunits. Recently, a non-GPCR GEF, RIC8, has been discovered as an important mechanism for G alpha regulation in animals. RIC8 is present in filamentous fungi, but is absent from the genomes of *Saccharomyces cerevisiae* and sequenced protists. Deletion of *ric8* in *Neurospora* leads to defects in polar growth and asexual and sexual development, similar to phenotypes observed for a mutant lacking the G alpha genes *gna-1* and *gna-3*. Additionally, activated alleles of *gna-1* and *gna-3* rescue many defects of *ric8* mutants, RIC8 interacts with GNA-1 and GNA-3 in the yeast two-hybrid assay, and *ric8* strains have greatly reduced levels of G protein subunits. Consistent with a role for RIC8 in asymmetric cell division in *C. elegans* and *Drosophila*, hyphae from *ric8* mutants have small cell compartments that contain nuclei that are small and morphologically abnormal. Taken together, our results support a role for RIC8 and G proteins in regulation of polar growth in *Neurospora*. RIC8 and G alpha proteins purified from *E. coli* will be used to test the G alpha GEF activity of RIC8 and GTPase activity of the G alpha proteins.

636. Silencing avirulance and effector genes in *Phytophthora sojae*.

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Avirulance (Avr) genes in *P. sojae* interact with Rps resistance genes in the soybean plant triggering a response responsible for the containment of the spread of the pathogen in the plant. The exact functions and molecular mechanisms of most Avr genes in *P. sojae* are largely unknown. Silencing Avr genes will help us to confirm the identity of the genes and to understand the role that each Avr gene plays during infection. Silencing can also test the possible effector functions of bioinformatically identified avirulence homolog (Avh) genes. Genes of interest are introduced into *P. sojae* for silencing by the protoplast/PEG method. Full or partial silencing is determined by real time reverse transcription PCR and avirulance tests in soybean plants.

637. Searching for answers: Site-of-action and structural requirements of the host-selective toxin Ptr ToxB.

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Pyrenophora tritici-repentis is a necrotrophic foliar pathogen responsible for the disease tan spot of wheat. Ptr ToxB (ToxB) is one of the proteinaceous host-selective toxins produced by this pathogen and is responsible for the development of chlorotic symptoms in susceptible cultivars. ToxB is encoded by a multicopy gene, *ToxB* (261 bp), whose expression results in an 87 amino acid (aa) pre-protein. This pre-protein contains a signal peptide of 23 aa and the remaining 64 aa encode the mature form of the toxin (6.5 KDa). There are no characterized motifs within ToxB to give clues to its functional site. An allele of *ToxB*, *toxb*, is found in non-pathogenic isolates and encodes an inactive protein. Construction of chimeric proteins containing *ToxB* and *toxb* coding regions, and site-directed mutagenesis based on the aa sequence differences have provided information on the structural requirements for ToxB activity. A Proteinase K protection assay using heterologously expressed ToxB has provided insight into the timing required for maximum symptom development and the possible site-of- action of ToxB. *Barley Stripe Mosaic Virus*-mediated transient systemic expression of ToxB and toxb supports data obtained from Proteinase K protection assay.

638. Metabonomics approach to study of macroconidiation and G protein signaling in *Neurospora crassa*.

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The filamentous Ascomycete *Neurospora crassa* senses environmental change through heterotrimeric G-protein signaling, similar to other organisms, including mammals. Heterotrimeric G-proteins, made up of G-alpha, G-beta and G-gamma subunits, interact with integral plasma membrane proteins known as G protein coupled receptors. In *N. crassa*, loss of the G-alpha protein GNA-3 leads to inappropriate conidiation in submerged culture. Since conidiation can be a response to nutrient starvation, the possibility that GNA-3 might be involved in nutrient sensing was explored. Utilizing proton-NMR, a metabonomics approach was used to create a metabolite fingerprint to compare amino acid levels and other metabolites in wild-type and *gna-3* deletion strains cultured under adequate and limiting carbon conditions. Statistical analysis showed that loss of *gna-3* does not significantly alter the metabolite fingerprint of *Neurospora*. However, individual compounds showed differences. For example, levels of the major carbon reserve trehalose decreased, while glucose levels increased in the *gna-3* deletion mutant. This suggests that the mutant is not able to correctly gauge environmental cues, and that GNA-3 may be involved in regulating carbon sensing in *Neurospora*.

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639. Ancient trans-specific polymorphism at pheromone receptor genes in basidiomycetes.

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In the majority of sexual organisms, reproduction occurs almost exclusively through the combination of distinct and alternate forms, called sexes or mating types. In some fungi, there can be dozens to hundreds of alternate alleles that determine compatible mating types. Such extensive polymorphism is expected to be maintained by balancing selection, and in extreme cases may give rise to trans-specific polymorphism. Here, we analyzed sequences of two pheromone receptors in the Microbotryum fungal species complex (Basidiomycota), which has only two alternate mating types. Several lines of evidence strongly suggest that the pheromone receptors are two allelic sequences acting to determine the alternate A1 and A2 mating types required for mating in Microbotryum. Phylogenetic trees of pheromone receptors in the Microbotryum species complex indicated a trans-specific polymorphism: the Microbotryum sequences from a given mating type were all more similar to the pheromone receptors of distantly related classes of fungi than to the alternate pheromone receptor in the Microbotryum species. A phylogenetic tree built using other known pheromone receptors from basidiomycetes showed that trans-specific polymorphism is widespread. The pheromone receptor alleles from Microbotryum appeared as the oldest, being at least 370 millions years old. This represents the oldest known trans-specific polymorphism known in any organism so far, which may be due to the existence of sex chromosomes, obligate sexuality, mitochondrial inheritance liked to the mating type and a highly selfing mating system in Microbotryum.

640. Fungal Biology lessons, a link between visual arts students and visually-impaired students.

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Participation in education and outreach activities is one of the most actual demands from the society to scientists, and it is especially important in countries like Mexico, where the number of scientist is relatively low. With the goal of stimulating the interest for science in young students, mainly elementary and high school kids, we designed and implemented a fungal biology hands-on workshop. Fungi are an ideal model for these activities because they can easily be related to everyday life. The workshop included experiments and activities to learn about fungal growth, morphology, development and metabolism, approached by a fun and interactive method. Because commercially available teaching materials are mostly visually oriented, a special challenge was encountered when these workshops were offered to visually impaired students. We solved this by inviting college students enrolled in visual arts majors to participate in the design and creation of three-dimensional tactile materials to teach various aspects of fungal biology. Additionally, multi-sensorial activities were included in the workshops, with the goal of compensating the lack of vision by stimulating other senses. Our observations showed that an all-win outcome was the main accomplishment of this work, encouraging the continuation of these type activities.

641. Evolution of effector genes in Phytophthora infestans and closely related species revealed by comparative Illumina genome sequencing.

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The Irish potato famine organism *Phytophthora infestans* is an economically important specialized pathogen that causes a destructive disease on *Solanum* plants. *P. infestans* and its clade 1c sister species, *P. andina*, *P. ipomoeae*, *P. mirabilis* and *P. phaseoli*, have most likely evolved by host jumps followed by adaptation to unrelated host plant species. The availability of multiple genome sequences of *P. infestans* and its sister species will provide unprecedented opportunities to understand the basis of genome evolution and host adaptation. We initiated Illumina genome sequencing and generated >50 million 35/36nt single reads for seven genomes representing 5 species, including *P. infestans* reference strain T30-4. To investigate overall abundance of single nucleotide polymorphisms, we aligned Illumina reads to the T30-4 supercontigs containing 18155 genes. We calculated the average rates of synonymous nucleotide substitutions per synonymous site (dS), and showed that it correlates with the current clade 1c phylogeny. Families of effector genes (RXLR, Crinklers, enzyme inhibitors) evolve faster than the remainder of the genome. We also detected a higher proportion of effector genes with no reads aligning to the reference sequence. The level of selection pressure was calculated revealing the effector genes under positive selection. In conclusion, our comparative genomics analyses will help to unravel the evolutionary fate of effector genes following a host jump and will ultimately advance our interpretation of the *P. infestans* genome sequence

642. Genetic structure of populations of *Phytophthora infestans* from China.

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Phytophthora infestans causes late blight and is the most devastating disease of potato in China. China grew more than 72 million tons of potato in 2007 in four major potato growing areas in the north and south. One hundred modern isolates of *P. infestans* collected from ten provinces including Beijing, Chongqing, Fuijan, Gansu, Hebei, Heilongjiang, Jilin, Inner Mongolia, Sichuan, and Yunnan between 1999-2004 were analyzed for mating type, metalaxyl resistance, mitochondrial DNA (mtDNA) haplotype, and a subset of 21 isolates were analyzed for allozyme genotype with *Glucose-6-phosphate isomerase* (*Gpi*), and *Peptidase* (*Pep*), restriction fragment length polymorphism fingerprint (RFLP) with the RG-57 probe, and DNA sequence variability from two nuclear (Ras, and Intron Ras) and two mitochondrial (P3 and P4) gene regions. All isolates tested were the A1 mating type except three isolates that were A2 and self fertile. Four allozyme genotypes including *Gpi* 86/100, *Pep* 92/100; *Gpi* 86/100, *Pep* 100/100; *Gpi* 100/100, *Pep* 100/100; and *Gpi* 100/111, *Pep* 100/100 were found. Three isolates from tomato and one isolate from potato were the US-1 genotype (*Gpi* 86/100, *Pep* 92 or 100/100, Ib mt DNA haplotype). A previously described genotype from Siberia called SIB-1 (*Gpi* 100/100, *Pep* 100/100, IIa mtDNA haplotype) was identified among isolates and was widely distributed in China in Gansu, Hebei, Heilongjiang, Inner Mongolia, and Jilin provinces in the north and Sichuan and Yunnan province in the south. A new genotype named CN-9 (*Gpi* 100/111, *Pep* 100/100, IIb mtDNA haplotype) was found only in the south of China. The remaining genotypes CN10-CN12 (*Gpi* 100/100, *Pep* 100/100, Ia mtDNA haplotype) had unique RFLP fingerprints and were named CN-9-CN12. We have examined potato leaves infected with *P. infestans* from herbarium collections (1901-1981) from China, Southeast Asia, India, Russia, and Australia. Twelve samples from China containing late blight lesions collected between 1938 and 1982 from Beijing, Hebei, Sichuan, Shanxi, Chongqing, and Yunnan were examined. The Ia mtDNA haplotype of *P. infestans* was found earlier in China than other mtDNA haplotypes and was likely the initial haplotype introduced. Samples collected by different researchers in China from tomato in 1938 in Kunming and from potato in 1940 in Chengjiang and Chongqing were the Ia mtDNA haplotype. In contrast, the earliest record of the Ib haplotype (US-1 genotype) of *P. infestans* in China was in 1952 on potato in the Sichuan region, in 1954 on potato in Hebei and in 1956 on tomato in Beijing. The Ib haplotype still occurs in the Beijing area on tomato, but the IIa haplotype is now dominant in modern populations. The predominant genotype of *P infestans* in China was SIB-1 (IIa haplotype) and identical to those from Siberia suggesting a Russian ancestry.

643. Identification of homoserine utilization genes on a supernumerary chromosome in *Nectria haematococca* Mating Population VI (MPVI) and their contribution to rhizosphere competency.

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The fungus *Nectria haematococca* MPVI is a habitat-diverse fungus and habitat-determining genes in this fungus can be found on supernumerary chromosomes called "conditionally dispensable" (CD) chromosomes. Previous work has shown that the *N. haematococca PDA1* CD chromosome, which carries genes for Pea Pathogenicity (*PEP* cluster), also carries genes for the utilization of homoserine, an amino acid found in large quantities in pea root exudates. Furthermore, a *N. haematococca* MPVI isolate that has a portion of this CD chromosome containing the genes for homoserine utilization (*HUT*) and lacked the *PEP* cluster is more competitive in the pea rhizosphere than an isolate without the CD chromosome. In this study, a combination of fungal transformation, insertional mutagenesis, and bioinformatics analyses were used to identify a cluster of five genes on the *PDA1* CD chromosome that was responsible for the HUT phenotype. One of the genes was only found in *N. haematococca* MPVI, another was a common fungal transcription factor and the other three had homologs involved in the synthesis of the amino acids methionine, threonine, and isoleucine, in which homoserine is an intermediate. Competition experiments between isolates with and without the *HUT* cluster demonstrated that the *HUT* cluster is responsible for the increased competitive ability of HUT^+ *N. haematococca* isolates in the pea rhizosphere. To our knowledge, this is the first study to identify a rhizosphere competency trait in a fungus and the results indicate that a dispensable portion of the *N. haematococca* genome carries two clusters of genes involved in habitat specialization.

644. The Phytophthora sojae avirulence locus Avr3c encodes a multi-copy RXLR effector that displays sequence polymorphisms among different pathogen strains

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Root and stem rot of soybean is caused by the oomycete Phytophthora sojae. The Avirulence (Avr) genes of Phytophthora sojae control race-cultivar compatibility. In this study, we identify the P. sojae Avr3c gene and show that it encodes a secreted RXLR effector protein of 220 amino acids. Sequence and transcriptional data were compared for predicted RXLR effectors occurring in the vicinity of Avr4/6, as genetic linkage of Avr3c and Avr4/6 was previously suggested. Mapping of DNA markers in a F2 population was performed to determine whether selected RXLR effector genes co-segregate with Avr3c phenotype. The results pointed to one RXLR candidate gene as likely to encode Avr3c. This was verified by testing selected genes by a co-bombardment assay on soybean plants with Rps3c, thus demonstrating functionality and confirming the identity of Avr3c. The Avr3c gene together with eight other predicted genes are part of a repetitive segment of 33.7 kb. Three near-identical copies of this segment occur in a tandem array. In P. sojae strain P6497, two identical copies of Avr3c occur within the repeated segments whereas the third copy of this RXLR effector has diverged in sequence. Virulent alleles of Avr3c that differ in amino acid sequence were identified in other strains of P. sojae. The Avr3c gene is expressed during the early stages of infection in all P. sojae strains examined. Overall, the results illustrate the importance of segmental duplications and RXLR effector evolution in the control of race-cultivar compatibility in the P. sojae and soybean interaction.

645. Septins: a corset for *Ustilago maydis*?

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Septins comprise a conserved family of proteins that are found primarily in fungi and animals. These GTP-binding proteins have several roles during cell division, cytoskeletal organization and membrane-remodelling events. One factor that is crucial for their functions is the ordered assembly of individual septins into oligomeric core complexes that, in turn, form higher-order structures such as filaments and rings.

Multiple septin genes have been identified in fungi and animals, and the number of septin genes in an organism varies widely, from 2 in *Carnorhabditis elegans* to 14 in humans. In *Ustilago maydis*, one septin has been described, Sep3 (Boyce *et al.* 2005).

We have identified three more septin genes; *sep1*, *sep2* and *sep4*. None of the septins is essential and their deletion phenotypes are very similar. Subcellular localisation analyses showed that all septins accumulate at the bud neck though different localisation patterns appear at other sites of the cell for each of the septins. Strickingly, one septin, Sep4, appears as long filaments running along the long axis of the cell. These filaments depend on the other septins but are independent of the microtubule and actin cytokeletons. Their putative role will be discussed.

Boyce KJ, Chang H, D'Souza CA, Kronstad JW. An *Ustilago maydis* septin is required for filamentous growth in culture and for full symptom development on maize. *Eukaryot Cell.* 2005 Dec;4(12):2044-56.

646. Role of different chitin synthases in the development of *Coccidioides posadasii*

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Coccidiodes immitis and *C. posadasii* are the causative agents of coccidioidomycosis, commonly known as Valley Fever. These fungal pathogens attack healthy mammals when spores are inhaled and cause respiratory symptoms similar to pneumonia. If the immune system of the infected individual does not respond, the fungus can spread throughout the body and eventually cause death. Valley Fever is endemic in the southwestern United States, along with parts of Mexico, Central and South America. There are 150,000 reported new infections per year, of which 100,000 occur in Arizona.

The cell wall of Coccidioides spp. is rich in chitin, thus chitin synthases (CHSes) are likely to play an important role in producing morphogenetic changes during the transition from saprobic (soil) to parasitic (lung) growth. Coccidioides has seven single CHSes that belong to classes I to VII (Mandel et al, 2006). We have generated mutant strains that each lack one CHS and are characterizing them. Preliminary results show that DCHS5 and DCHS7 have a strong phenotype consisting of aberrant morphologies during mycelial growth, with thickening of the cell wall, balloon-like structures and a reduced growth rate. The DCHS5, in addition, produces little or no conidia. DCHS7 is sensitive to Calcofluor White, Congo Red and SDS compared to wild-type. Interestingly, this mutant is highly resistant to nikkomycin Z (nikZ), an antifungal compound that specifically targets CHSes. Our hypothesis is that CHS7 is necessary for part of the integrity of the cell wall in *Coccidioides* and it is also the target of nikZ. Expression of the CHSes in different mutant backgrounds will be presented.

647. Fast evolution of a set of non self recognition genes in *Podospora anserina*.

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Vegetative incompatibility is a non self recognition process ubiquitous in fungi that occurs after anastomosis between isolates of unlike genotypes. VI is triggered by the co-expression of incompatible alleles of specific recognition genes called *het* and leads to the destruction of the fusion cell. *het* genes are unusually polymorphic and unravelling mechanisms for genesis/maintenance of polymorphism is key to understanding VI.

In *Podospora anserina*, co-expression of incompatible *het-C*/*het-D* or *het-C*/*het-E* or *het-R*/*het-V* alleles results in VI. *het-D*, *het-E* and *het-R* belong to a large gene family encoding for three domain proteins with an N-terminal HET domain effecter of cell death, a central NACHT domain binding to GTP, and a variable C-terminal WD domain whose sequence defines allelic specificity. The WD domain is made of a variable number of tandem repeats of WD40 units that assemble in a b-propeller fold involved in protein-protein interactions.

By combining genomic and experimental approaches we demonstrate a highly dynamic process promoting diversification of the WD40 unit repertoire. Three evolutionary forces combine to promote a fast evolution of the WD40 repeat sequences: a high mutation load of the repeat sequences due to high copy number and Repeat Induced Point mutation, concerted evolution that results in WD40 sequence exchanges between members of the family, and positive selection acting at four positions involved in protein protein interactions. Genetic instability of the WD repeat domains increases diversification of the WD40 repertoire. Fast evolution of the WD40 repeat sequences is associated with fast evolution of the *het-c* partner in incompatibility suggesting their co-evolution.

648. A C-terminal motif in RPD3-type histone deacetylases of filamentous fungi is essential for growth and development of *Aspergillus nidulans*.

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Acetylation of the N-terminal tails of core histones is crucial for the regulation of a large number of genes in eukaryotic organisms. Although filamentous fungi have significantly contributed to our understanding of gene regulatory mechanisms, only little is known about the functions of histone acetyltransferases (HATs) and histone deacetylases (HDACs), which are responsible for a balanced acetylation status of histone tails. Further, increasing evidence suggests that HDACs affect the regulation of genes involved in pathogenicity, stress response, and production of secondary metabolites like antibiotics or toxins of fungi. Here we show that depletion of RpdA, a class 1 HDAC of *Aspergillus nidulans*, leads to a drastic reduction of growth and sporulation of the fungus. Functional studies revealed that a short C-terminal motif unique for RpdA-type proteins of filamentous fungi is required for the catalytic activity of the enzyme and consequently, cannot be deleted without affecting the viability of *A. nidulans.* Moreover, evidence is provided that this motif is also essential for other filamentous fungi. Thus, the C-terminal extension of RpdA-type proteins represents a promising target for fungal specific HDAC-inhibitors that might have potential as new antimycotic compounds with clinical and agricultural applications.

649. Putative autophagy protein Atg13 affects the morphology of the filamentous fungus *Aspergillus nidulans*

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In both natural and man-made environments, filamentous fungi commonly experience nutrient starvation and adapt to this condition by modifying either their physiology, their morphology, or both. We have hypothesized that during nutrient deprivation, the non-selective degradation process known as autophagy plays an important role. Autophagy is ubiquitous in eukaryotic cells and its components are strongly conserved from yeast to man. Here, we study the effect of autophagy inducing conditions (carbon and nitrogen starvation, rapamycin exposure) on an An*atg13* deletion mutant.
In rich growth medium there is no observable difference in growth and morphological development between the An*atg13* deletion mutant and its isogenic parent (TN02A3). In contrast, autophagy inducing conditions lead to reduced vacuolation and significantly fewer vacuolar contents, suggesting the An*atg13* deletion impairs autophagy to a significant degree. Metabolic activity, assessed via XTT assay, shows behavior consistent with atg13 playing a role in autophagy - carbon and nitrogen starvation lead to significantly reduced activity in the mutant whereas rapamycin exposure does not. Quantitative studies were carried out to determine growth and branching rates. Surprisingly, the deletion mutant shows a significant reduction in both growth and branching rates during growth in complete minimal medium, implying autophagy may be active even in the absence of starvation. Perhaps most interesting was the impact of the An*atg13* deletion on morphology - there was no significant change in branching rate in any of the various growth environments employed. In contrast, the isogenic parent showed a significant reduction in branching rate during starvation. These results imply that autophagy, or the Atg13 protein, may play a role in regulation of hyphal branching rate.

650. Aspergillus fumigatus developmental regulator medA also governs virulence, adherence, and interactions with host cells.

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Regulatory programs governing fungal development also modulate virulence in a variety of medically important fungi. In *Aspergillus nidulans* the developmental modifier MedA governs conidiation, but has not been studied in virulence. We therefore identified and disrupted MedA in the pathogenic species *Aspergillus fumigatus*. Disruption of *medA* resulted in dramatically reduced conidiation, although differences in conidiophore morphology and gene expression analysis suggest that MedA governs conidiation through different pathways in *A. fumigatus* compared to *A. nidulans*. MedA deficient strains were also impaired in several virulence-associated phenotypes in vitro including biofilm production; adherence to pulmonary epithelial cells, endothelial cells and fibronectin; induction of pulmonary epithelial cell damage; and stimulation of pro-inflammatory cytokine mRNA and protein expression by epithelial cells. Consistent with these in vitro phenotypes, the *A. fumigatus medA*-null mutant strain also exhibited reduced virulence in both an invertebrate and a mammalian model of invasive aspergillosis. Collectively these results suggest that the downstream targets of *A. fumigatus* MedA mediate virulence. Identification of these downstream effectors using whole genome transcriptional analysis is ongoing.

651. Genetic networks for the functional study of the genome of the white rot basidiomycete *P. ostreatus* and its application to biotechnological processes.

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The high throughput analytical techniques used in genome, proteome and metabolome studies produce large sets of data that must be studied using appropriate tools. The construction of networks linking different genetic elements and/or functions make it possible to obtain an integrated view of the cell molecular biology and will eventually help us to predict complex phenotypes from molecular data. Genetic networks can be constructed using different types of data such as genes involved in the control of complex phenotypic traits, genes controlling global gene expression, genetic elements involved in the same metabolic process, gene products interacting physically between them. The connections linking these genetic elements in the network reflect the genetic, physical and/or functional interaction among them. All these networks share common properties and reflect the different layers of the cell's complexity. In the years 2007 and 2008 the Department's of Energy Joint Genome Institute has sequenced the whole genome of the white rot edible basidiomycete *Pleurotus ostreatus* (http://www.jgi.doe.gov/sequencing/why/50009.html). This will be the first edible fungus whose genome to be sequenced. *P. ostreatus* is also interesting because of its lignin degrading activity that differs from those of the other white-rot model *Phanerochaete chrysosporium* and of the brown-rot model *Postia placenta.* We are using some of the network analysis strategies discussed above to determine *P. ostreatus* genome regions involved in the control of lignin degradation processes aimed at the use of this fungus for wood and straw biorefinery processes (http://www.unavarra.es/genmic).

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652. Telomere organization in the ligninolytic basidiomycete *Pleurotus ostreatus*

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Telomeres are structural and functional chromosome regions essential for the cell cycle to proceed normally. They are, however, difficult to map genetically and to identify in genome-wide sequence programs because of their structure and repetitive nature. We have studied the telomeric and subtelomeric organization in the basidiomycete *Pleurotus ostreatus* using a combination of molecular and bioinformatic tools that have permitted to determine 19 out of the 22 telomeres expected in this fungus. The telomeric repeating unit in *P. ostreatus* is TTAGGG, and the number of repetitions of this unit ranges between 25 and 150. The mapping of the telomere restriction fragments to linkage groups 6 and 7 revealed polymorphisms compatible with those observed by Pulse Field Gel Electrophoresis separation of the corresponding chromosomes. The sub-telomeric regions in *Pleurotus* contain similar genes to those described in other eukaryotic systems. It is especially relevant the presence of a cluster of laccase genes in chromosome 6, and a bipartite structure containing a Het-related protein and an alcohol dehydrogenase; this bipartite structure is characteristic of the Pezizomycotina fungi *Neurospora crassa* and *Aspergillus terreus*. As far as we know, this is the first report describing the presence of such structure in basidiomycetes and the location of a laccase gene cluster at the subtelomeric regions where usually map, among others, species-specific genes allowing the organism a rapid adaptation to the environment.

653. Analysis of *Botrytis cinerea* putative apoptotic genes *BcBIR* and *BcNMA*

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Apoptotic-like programmed cell death (PCD) is part of fungal response to stresses and it is also involved in regulation of certain developmental processes such as ageing and reproduction. Fungal homologs of mammalian apoptotic genes have been identified, supporting conservation of core apoptotic machinery in fungi. Our goal is to gain knowledge on the regulation and role of apoptosis in fungi and particularly to determine possible involvement in pathogenic development. Here we describe the isolation and partial characterization of two putative apoptotic genes from the gray mold fungus *B. cinerea*; *BcBIR*, a homolog of *Saccharomyces cerevisiae* IAP-like gene *BIR1*, and *BcNMA*, a homolog of *S. cerevisiae NMA111,* which is a homolog of the human *Omi/HtrA*. IAPs (inhibitor of apoptosis proteins) prevent apoptosis in human through inhibition of caspases. Omi/HtrA is a mitochondria residing serine protease that degrades IAPs thereby promoting apoptosis.
B. cinerea homologs of both genes were identified by BLAST search using the human and yeast genes as queries. *B. cinerea* was transformed with knockout and over expression cassettes of the *BcBIR* and *BcNMA* genes. Homokaryons of D*bcbir* knockout could not be generated and we concluded that the gene is essential. Similarly, we could not purify homokaryons of *BcNMA* over expression strains, suggesting a lethal effect on the fungus. The *BcBIR* over-expression and the D*nma* knockout strains were characterized. *BcBIR* over-expression isolates accumulated more biomass in liquid cultures, had reduced sensitivity to nutrient stress, and were slightly more virulent than wild type. When exposed to salt stress or at stationary phase they accumulated less ROS than wild type and retained normal nuclear morphology. The D*bcnma* had similar, though not identical phenotypes. Differences from the *BcBIR* over-expression strains included reduced germination and slight reduction in virulence. Protein localization and possible interaction between the two proteins were also studied and will be described. Overall, our results suggest that both proteins are associated with response to stress and constitute part of the fungal apoptotic regulatory network.

654. Proteomic analysis of secreted proteins of the chestnut blight fungus *Cryphonectria parasitica*

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In addition to its biological control potential, the hypovirus-infected hypovirulent chestnut blight fungus *Cryphonectria parasitica* has also been used as a model system to dissect the fundamental biological process of the fungus, e.g. signal transduction and virulence regulation. Hypovirus infection globally alters fungal gene transcription profile and phenotype that typically include reduced virulence and suppressed production of pigment, sexual and asexual spores, and certain extracellular enzymes. By taking the proteomic approach, we analyzed the secreted proteome of the wild type *Cryphonectria parasitica* strain EP155 and the hypovirus-infected strain EP713. Proteins secreted into the liquid medium were analyzed at different time points with two-dimensional gel electrophoresis (2-DE) followed by MALDI-TOF/TOF identification. Two hundred and fifty protein spots were found on 2D gels and about 40% of which have been identified. Most of the proteins identified are enzymes involved in the metabolism of sugar, protein, nucleic acid, and lipid, e.g. xylanase, glyoxalase, dehydrogenase, acetyltransferase, protease, amidohydrolase, peptidase, endoribonuclease, lipase, and esterase. Other key enzymes such as oxidase, reductase, superoxide dismutase, peroxiredoxins, isomerase, and transaldolase, were also found. Alterations in the secreted protein expression pattern after hypovirus infection were observed. *Corresponding author

655. Transcriptional and post-transcriptional regulation of chitin synthesis in *Candida albicans.*

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Chitin synthesis is essential for growth, viability and rescue form cell wall stress. In *Candida albicans*, chitin synthesis is achieved by four isoenzymes, Chs1, Chs2, Chs3 and Chs8 and their expression is regulated via multiple signalling pathways. Promoter dissection experiments showed that PKC cell wall integrity pathway may operate through RLM1-elements in the *CHS2* and *CHS8* promoters, but that promoter sequences that respond to the Ca^{2+}/calcineurin and HOG signalling pathways in *S. cerevisiae* did not directly regulate transcription of *CHS2* and *CHS8*. An analysis of the *C. albicans* phosphoproteome revealed that CaChs3 is phosphorylated and that mutation of this site showed that both phosphorylation and de-phosphorylation are required for the correct localisation and function of Chs3. The kinase Pkc1 was required to target to Chs3 to zones of polarised growth, but not to sites of septa. All four Chs enzymes localize to sites of septation. Interestingly septa could be formed in a range of *chs* mutant backgrounds if the cell wall salvage pathways were activated. This implies that septation can be achieved via redundant pathways under conditions of cell wall stress.

656. Screening for the role in plant disease of *Hyaloperonospora arabidopsidis* effectors.

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Several plant pathogens can secrete effectors molecules that once delivered into the host could manipulate plant defences allowing the pathogen to complete its life cycle. As an obligate biotroph, the oomycete *Hyaloperonospora arabidopsidis* (*Hpa*) that colonizes *A. thaliana*, requires living host tissue for growth and reproduction. These effectors are predominantly small secreted proteins containing a signal peptide and the RxLR EEr motifs. Bioinformatics analysis of the *Hpa* Emoy2 race genome was carried out and around 140 potential effectors were identified. Selection of candidates expressed during the infection process was done by sequencing ESTs of a 3dpi cDNA library and short reads of a 7dpi sequence tag library (www.illumina.com). Together with the ERA-PG Consortium we have cloned 102 of these potential effectors and are studying them for (i) Functions in virulence or avirulence in *A. thaliana* (ii) Genetic variation in the host for their recognition. For this, we use the EDV system of *Hpa* effectors delivery through *Pseudomonas syringae* TTSS (*Pst)* (Sohn et al., Plant Cell 2007). Using different *Psts* we could assess if a given *Hpa* effector could increase/decrease bacterial growth "*in planta*" (*Pst*-LUX), and if can suppress PAMP triggered immunity (PTI) by monitoring callose deposition suppression when delivered through *Pst* deltaCEL. Preliminary results indicate that around 80% of the effectors tested can enhance bacterial growth on at least one of 12 *A.t.* accessions tested while 18% of them can restrict bacterial growth, and some of these provoke HR-like lesions on *A.t* . Also, 50% of the effectors are able to suppress callose accumulation indicating PTI suppression. Mixed infections using sets of *Psts* carrying different effectors are also being developed and results will be presented.

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657. Development of a method of measuring the activity Laccase segregated by monokarions of *Pleurotus ostreatus* during the process of biological pretreatment of lignocellulose for the production of bioethanol.

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The utilization of low-value substrates such as lignocellulosic wastes offers a great potential for reducing the production costs of bioethanol. The biological process of bioethanol production using lignocellulose as feedstock requires its delignification to liberate the cellulose and hemicellulose from their complex with lignin. *P. ostreatus* produces various lignolytic activities that act in this process. One of them is the laccase. In this work, we have set a protocol for measuring the laccase activity secreted in solid cultures performed on poplar (*Populus alba*) sawdust, we have followed its evolution over the culture time and we have studied its variation in different
strains. We show here (1) that the extractable laccase activity was higher when the cultures were inoculated with mycelium resuspended in water than when the mycelium was resuspended in complex media, (2) that different monokaryotic strains displayed different profiles of laccase activity over the culture time, and (3) that there is evidence suggesting the presence of an inducer of the secretion laccase activity in the sawdust that can be extracted form the wood. Moreover, monokaryons mk009 and mk116 displayed the highest secreted laccase activity and they are, therefore, the best suited ones for the development of a biological pretreatment of
lignocellulosic substrates for the industrial production of bioethanol. These two monokaryons showed different patterns of temporary expression of laccase activity. Finally, monokaryon mk009 produces laccase activity earlier in the culture and maintains this activity over all the culture time analyzed.

658. DNA barcoding of *Septoria* species from leaf spots and stem cankers of Poplar in British Columbia, Canada to assess risk of spread.

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Widespread use of hybrid poplar in north-central and northeastern North America is limited by occurrence of a leaf spot and canker disease caused by the haploid Coelomycete fungus Septoria musiva (teleomorph Mycosphaerella populorum). Molecular marker analyses previously allowed us to document the occurrence of genetically differentiated *S. musiva* subpopulations in north-central and northeastern North America, with both asexual and sexual recombination contributing to the genetic structure. With the extension of poplar cultivation and the use of hybrid poplars, stem cankers have recently been reported in new bioclimatic domains in the province of Québec and in the Fraser valley in British Columbia (BC) previously considered to be S. musiva-free. By harvesting and mapping infected poplars and conducting DNA barcoding to assess *Septoria* species distribution we can evaluate the risk of damage of this plant pathogen in these newly infected area and assess the potential for eradicating the pathogen. DNA barcoding of poplar leaves harvested in the Fall of 2008 using ITS rRNA indicated that the native *Populus trichocarpa* is almost exclusively infected by the native *S. populicola* while hybrid poplars are infected by the non-native *S. musiva*. Although further sampling is necessary to confirm these trends, it appears that *S. musiva* is mostly restricted to planted hybrid poplars and do not display a propensity to spread to native *P. trichocarpa*.

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659. The high affinity sucrose transporter Srt1 from *Ustilago maydis* is required for pathogenic development.

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The smut fungus *Ustilago maydis*, a ubiquitous pathogen of maize, is highly adapted to its host, avoiding aggressive virulence strategies that harm the plant. Although fungal hyphae traverse the plant cells, there is no apparent host defense response and the cells remain alive. *U. maydis* infection leads to the formation of plant tumors that show the characteristics of typical sink tissues, resulting in transport of sucrose by the plant to the area of fungal proliferation.
Despite the availability of sucrose, all fungal transporters identified so far in symbiotic or pathogenic interactions are specific for monosaccharides. We have now identified a novel type of plasma membrane transporter, Srt1, that transports sucrose with high specificity. Deletion of Srt1 in *U. maydis* reduces fungal virulence and triggers a plant response reaction, emphasizing the central role of sucrose-uptake during biotrophic development. The extremely high affinity of Srt1 to sucrose may not only allow the efficient competition with plant transporters at the plant/fungal interface, but also prevent a plant response triggered by increased levels of extracellular monosaccharides as glucose and fructose caused by pathogens.

660. Identification and analysis of differentially expressed Heterobasidionparviporum genes during natural colonization of Norway spruce

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To identify differentially expressed genes of the white-rot fungus Heterobasidion parviporum subtractive cDNA libraries were constructed using suppressive subtraction hybridization (SSH) technique with RNA extracted from an advanced stage of decay area and from colonization front next to the reaction zone of the stem of a mature Norway spruce trees. Besides cytochrome P450s and proteins with unknown function, the SSH libraries constructed contained genes involved in basic cellular processes, andcell wall degradation. To examine the role of selected candidate genes three trees, showing a variable degree of wood decay, were used for real-time RT-PCR profiling of candidate genes. In the decay transition areas the study revealed activity centers that showed remarkable similarity in the transcript profiles of monitored genes

661. Identifying virulence factors in the fungal pathogen, *Histoplasma capsulatum*

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Phagocytic immune cells such as macrophages possess a considerable arsenal of microbicidal mechanisms. Nonetheless, there are a significant number of pathogens which evade these defenses to survive and replicate within host phagocytes. Studying the myriad survival strategies utilized by these pathogens provides insight into disease pathogenesis, as well as host cell biology.*Histoplasma capsulatum* is a dimorphic fungal pathogen that is well adapted to survive within the macrophage. The organism exists in the environment as a filamentous mycelial form that is easily aerosolized and inhaled by the host. Inside the host, the mycelial form converts into a pathogenic yeast form. *H. capsulatum* yeast are able to survive and replicate within host macrophages, eventually causing cell lysis. In a healthy host, infection by *H. capsulatum* is limited by cell-mediated immune response. However in an immunocompromised host, infection by *H. capsulatum* leads to a disseminated and often fatal disease.
The molecular strategies employed by *Histoplasma* to survive and replicate inside macrophages are not well understood. Only two virulence factors, Yps3 and Cbp1, have been identified in the *H. capsulatum* strain G217B. To identify additional mechanisms contributing to the survival and virulence of *H. capsulatum,* our lab employed a high-throughput insertional mutagenesis to identify 47 *H. capsulatum* mutants defective in macrophage lysis. We predicted that some of these mutants might fail to survive within macrophages, whereas others might survive and replicate within macrophages but fail to lyse them. At least two of these mutants are disrupted in the *CBP1* gene, providing validation that the screen identified genes required for macrophage colonization and lysis. The remainder of the lysis defective (*ldf*) mutants display moderate to severe macrophage lysis defects as gauged by a quantitative cell-lysis assay. We have determined which of the mutants are defective for survival within macrophages, and have also identified the site of insertion for the majority of the mutants. We are currently determining which of the mutants have virulence defects in the mouse model of histoplasmosis.

662. Nuclear-cytoplamic shuttling of FRQ is essential for clock function in *Neurospora crassa*

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Circadian clocks are self-sustained cellular oscillators that organize temporal expression of a large number of genes in many organisms. Negative transcriptional/translational feedback loops constitute the core of such clocks. In *Neurospora crassa* the circadian clock protein Frequency (FRQ) feedback-regulates its own expression by inhibiting its transcriptional activator, White Collar Complex (WCC) in the nucleus. However only a minor fraction of FRQ is localized in the nucleus. To analyze the nucleo-cytoplasmic distribution of FRQ we have developed a biochemical assay based on rapamycin-induced complex formation between the FK506 binding protein (FKBP) and the FKBP-rapamycin binding domain of mTor (FRB). FKBP and FRB were fused to histone H1 and FRQ, respectively. Incubation of the corresponding Neurospora strain with rapamycin resulted in rapid relocalization of cytoplasmic FRQ to the nucleus. The data demonstrate that FRQ is shuttling between compartments. The apparent cytosolic enrichment of FRQ reflects the equilibrium between rapid nuclear import and export. We have identified a nuclear export signal in FRQ. Deletion of this signal results in arrhythmic growth, suggesting that nucleo-cytoplasmic shuttling of FRQ on a non-circadian time scale is essential for the function of the circadian clock.

663. Cbp1 is required for lysis of host macrophages by the fungal pathogen *Histoplasma capsulatum*.

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Histoplasma capsulatum is a fungal respiratory pathogen that infects mammalian host macrophages. Successful infection is characterized by intracellular replication of the fungus followed by macrophage lysis. To date, however, very little is known about how *Hc* interacts with the macrophage to trigger host cell death. The secreted factor Cbp1 has previously been shown to be required for host colonization and virulence. Using a *cbp1* insertion mutant, we show that *CBP1* is dispensible for *Hc* growth in bone marrow derived macrophages (BMDMs), but not for BMDM lysis. The *cbp1* mutant replicated to higher levels within macrophages than wild-type *Hc*, but the mutant was incapable of triggering macrophage lysis. Complementation with the CBP1 gene restored the ability of the mutant cells to lyse host macrophages. To further understand the role of Cbp1 during infection, we examined the transcriptional profile of macrophages infected with wildtype *Hc* or the *cbp1* mutant. We identified a host transcriptional signature that was induced specifically by live wildtype *Hc* in a Cbp1-dependent manner. These data suggest that Cbp1 promotes a specific transcriptional program that correlates with lysis of host macrophages.

664. The *Histoplasma capsulatum* virulence factor cbp1 mediates a unique transcriptional signature in host macrophages associated with lysis

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The fungal pathogen *Histoplasma capsulatum* replicates within macrophages, subverting the host immune response and eventually leading to macrophage lysis. To better understand the macrophage molecular response to infection with *H. capsulatum* yeasts, we have performed whole-genome expression profiling on macrophages infected with *H. capsulatum*, which has identified a unique transcriptional signature not observed upon infection with bacterial pathogens. This transcriptional signature, which we refer to as the *Histoplasma* response cluster, contains genes indicative of an integrated stress response and is induced in macrophages infected with wild-type *H. capsulatum* yeasts, but not with UV-treated yeasts or mutants that fail to lyse host macrophages. A detailed examination of the kinetics of macrophage lysis and gene expression shows that expression of the *Histoplasma* response cluster precedes lysis. Interestingly, an *H. capsulatum* strain harboring a mutation in the virulence factor cbp1 that fails to lyse macrophages also fails to induce the *Histoplasma* response cluster, despite its ability to replicate intracellularly and activate an inflammatory response identical to that of wild-type *H. capsulatum*. Future experiments are aimed at elucidating the potential role of this gene expression program in mediating macrophage lysis during infection.

665. Functional annotation of Histoplasma capsulatum using tiling microarrays

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Histoplasma capsulatum is a dimorphic fungal pathogen that is thought to infect approximately 500,000 individuals per year in the U.S. Normally found in the mycelial form in soil at 25C, H. capsulatum converts to the pathogenic yeast form at 37C in the lungs of the host. The elucidation of H. capsulatum pathogenesis and biology will be greatly aided by the recent genomic sequencing of H. capsulatum strains G217B and G186AR at the Genome Sequencing Center (GSC) at Washington University in St. Louis and strains NAm1 (WU24), G186AR, H143, and H88 at the BROAD institute. The GSC used a variety of tools to generate a set of predicted genes for G217B and G186AR; however, these predictions are based on limited experimental data. Our goal is to generate a genome-wide functional annotation for H. capsulatum. To determine experimentally the full transcriptional repertoire of the genome, we have generated a set of 93 unique tiling microarrays composed of 50 bp oligomers spanning the non-repetitive regions of the G217B genome. To generate a diverse RNA sample, we prepared RNA from yeast-form cells grown under a variety of conditions (including early, middle, and late logarithmic growth, stationary phase, heat shock, oxidative stress, sulfhydryl reducing stress, and a range of media). The tiling microarrays were hybridized with fluorescently labeled cDNA generated from a pool of the yeast samples. The resultant data gives a measure of expression level as a function of genome position, and thus identifies the location and boundaries of expressed genes. 62% of the non-redundant genome was detected as transcribed, corresponding to 70% of the non-repeat predicted genes. 3% of the detected transcripts were novel with respect to the predicted gene set. Of 94 novel transcripts chosen for validation, 79 could be detected by RT-PCR. A web-accessible database linking the results of this study to additional data from Histoplasma and related fungi is available at http://histo.ucsf.edu.

666. Using ChIP-chip to characterize Ca^{++}/calcineurin transcription factor binding sites in *Magnaporthe oryzae*

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Significant progress has been made to define the central signaling networks in many organisms, but collectively we know little about the downstream targets and the genes they regulate. To reconstruct the regulatory circuit of calcineurin signal transduction via *MoCRZ1*, a *Magnaporthe oryzae* C2H2 transcription factor activated by calcineurin dephosphorylation, we are identifying the DNA motifs to which this protein binds *in vivo* using chromatin immunoprecipitation and microarray (ChIP-chip). The mocrz1 mutant showed impaired growth in the presence of Ca^{++} and has a pathogenicity defect mainly due to the inability to penetrate. We developed a non-coding region array of the *M. oryzae* genome in collaboration with NimbleGen technology. MoCRZ1 bound DNA fragments from eGFP tagged strain at the C terminus under its native promoter was immunoprecipitated with antiGFP antibody. More than 340 genes were identified as candidate targets with genes encoding calcium signaling components, and those involved in small molecule transport, ion homeostasis, and cell wall synthesis/maintenance being highly represented. To support ChIP-chip results and to obtain a global view of gene regulation by calcium signaling, a comprehensive microarray gene expression studies were also conducted. These data will provide support for remodeling calcium/calcineurin regulated signal transduction circuits controlling development and pathogenecity of this plant pathogenic fungus.

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667. GliT, a gliotoxin reductase, protects *Aspergillus fumigatus* against the harmful effects of gliotoxin.

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Gliotoxin, an epipolythiodioxopiperazine (ETP)-type toxin (326 Da) containing an essential disulphide bridge, plays a major role in mediating the virulence of the human pathogen, *Aspergillus fumigatus*. Gliotoxin toxicity in mammalian cells is generally enabled by direct inactivation of essential protein thiols as well as redox cycling, leading to hydrogen peroxide formation. In *A. fumigatus*, enzymes involved in gliotoxin biosynthesis are located within a coordinately-expressed, multi-gene cluster. Here we report the functional characterisation of a putative thioredoxin reductase encoded by *gliT* within this gene cluster. Expression of *gliT* is subject to regulation by the transcriptional activator GliZ. Moreover, expression of *gliT* is up-regulated GliZ-independently upon treatment with gliotoxin and in a development-dependent, but BrlA- and AbaA-independent, manner. MALDI ToF MS confirmed recombinant Escherichia coli-mediated expression of GliT (molecular mass of 38kDa). Purified, native GliT can act as a gliotoxin reductase and can catalyse the NADPH-mediated reduction of oxidised gliotoxin. Deletion of *gliT* is detrimental for growth only in the presence of exogenously added gliotoxin, which can be cured by supplementation with reduced glutathione. GliT is localised in the cytoplasm and in the nucleus. GliT is not essential for virulence of *A. fumigatus* in larvae of the greater wax-moth *Galleria mellonella*. The potential autoprotective role of GliT was investigated further by heterologous expression of *gliT* in *Aspergillus nidulans*. And indeed, GliT conferred resistance to gliotoxin, making it a valuable tool for transformation of fungi lacking an ortholog of *gliT*.

668. Biosynthesis of aspergiolide A, a novel antitumor compound by a marine-derived fungus Aspergillus glaucus via the polyketide pathway

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Aspergiolide A, a novel antitumor compound, was produced by a marine-derived filamentous fungus *Aspergillus glaucus*. The biosynthesis of it was unambiguously determined by feeding experiments using [l-13C]sodium acetate, [2-13C]sodium acetate, and [1,2-13C2]sodium acetate precursors followed by 13C NMR spectroscopic investigation of the isolated products. Analysis of the patterns of 13C-enrichment revealed that all 25 carbon atoms in skeleton of aspergiolide A were derived from labeled acetate. And among them, 12 carbon atoms were labeled from the carboxylic group of acetate, while the other 13 carbon atoms were labeled from the methylic group of acetate. Besides, the labeling pattern of [1,2-13C2]sodium acetate feeding experiment demonstrated that 12 intact acetate units were incorporated in aspergiolide A by polyketide pathway.

669. Transcriptome sequencing to understand effector evolution in the Phytophthora infestans species cluster.

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The highlands of Central Mexico are the center of origin of the potato late blight pathogen Phytophthora infestans. They are also home to several species closely related to P. infestans, namely P. mirabilis on Mirabilis jalapa and P. ipomoeae on Ipomoea longipedunculata. These species are thought to have evolve by host-jump followed by adaptation and specialization on distinct host plants. P. infestans secretes a large repertoire of effector proteins that evolve rapidly through birth-and-death evolution and typically exhibit adaptive selection. Our aim is to to identify candidate effectors that show species-level polymorphisms that can be related to host adaptation. We applied Illumina technology to sequence the transcriptomes of P. mirabilis and P. ipomoeae represented in normalized cDNA libraries constructed from mixed developmental stages including mycelia and germinated cysts. De novo sequence assembly revealed a novel RXLR effector present in both P. ipomoeae and P. mirabilis species but annotated as a pseudogene in the P. infestans T30-4 reference genome due to a 5-bp deletion. We found that transient expression of functional alleles resulted in suppression of plant immunity and we are currently expanding these experiments to the various hosts. Our study highlights the value of comparing transcriptomes from closely related species to identify candidate effectors in species with no prior gene sequence information. We now aim to connect the discovered genes to effector activities to reveal a putative role in host adaptation.

670. Proteomics Analysis of Iron Deprivation in *Cryptococcus gattii*

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Iron is a metal with ubiquitous distribution and the competition by this metal between host and pathogen is an important aspect for disease establishment. *Cryptococcus gattii* is an encapsulated yeast, which causes cryptococcosis in immunocompetent individuals. Iron levels and the expression of virulence factors in Cryptococcus species such as production of capsule and melanin and the ability to grow at 37°C may be related. In *C. gattii* until now there is no reports involving iron and expression of virulence factors. In this study, we examine the differential protein expression and the global protein expression in response to iron deprivation by using 2D-PAGE and multidimensional protein identification technology (MudPIT). The major focus is to identify proteins possibly involved with pathogen-host interactions. Our model strain is *C. gattii* R265, which was the cause of an epidemic outbreak in Vancouver Island, Canada. The treatment with low-iron medium (LIM) or iron-replete medium (LIM + FeHEDTA) was done by incubating of 5 x 10^7 cells mL^{-1} in these media for 12 hours at 37°C/ 200rpm. Comparison of the expression profile of the cells exposed or not to iron revealed a change of expression levels in at least 28 proteins, analyzed with PDQuest software. Proteins with differential expression were excised and are being identified using peptide mass fingerprinting obtained by Matrix-assisted laser desorption/ionization (MALDI-TOF). MudPIT will be applied to obtain the proteome of *C. gattii* R265 grown with or without iron. Financial support: FINEP, CNPq, CAPES

671. Molecular characterisation of peroxisome transport in filamentous fungi.

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Eukaryotic cells contain a large number of functionally distinct membrane-bound compartments, including the endoplasmic reticulum, mitochondria and peroxisomes. The position of these organelles is not fixed and maybe dependent on cell type, organisms or in response to external stimuli. How organelles are distributed in a cell is one of the central questions in cell biology. In this research the aim is to understand the migration, division and proliferation of peroxisomes. In filamentous fungi peroxisomes play an essential role in that they carry out reactions such as the glyoxylate cycle in the absence of the glucose. How this organelle populates the growing hyphae is a commonly asked question and is investigated in this research through looking at the key components involved in movement. For example, in animal cells, microtubules mediate long-range transport of organelles whereas actin mediates short-range transport and anchorage at the cell periphery. On the other hand, in bakers yeast and plants, organelle transport relies mainly on an actin-dependent process. In *Aspergillus nidulans*, addition of benomyl results in disassembly of microtubules, preventing both local movement and movement of peroxisomes out of the spore into the germ tube. As well as looking at the movement, we also investigated peroxisomal biogenesis. We used the promoter replacement approach to control expression of the three important biogenesis genes, PEX3, PEX19 and PEX16. Removing each of these genes by repression of the conditional promoter, *alcA* results in the loss of peroxisomes. Replacing these genes by switching the promoter on restores the peroxisomes. To further investigate if peroxisomes appear *de novo* or by division, pulse chase experiments were carried out. It was found that peroxisomes multiply by division. It is only in situations when essential gene products are lost and regained that the peroxisomes appear *de novo*.0

672. Analysis of a fungal pathogen of snakes reveals a potentially novel genus in the Onygenales

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Molecular and morphological analysis of a fungus isolated from two snakes brought to the Penn State Veterinary School revealed an apparently novel taxon within the Onygenales, closely related to *Coccidioides*. The snakes, both from a wildlife rehabilitation center, exhibited weight loss and external skin lesions, and both died from the infection. Necropsy revealed external lesions that extended through the body wall, where they were raised and proliferative. White nodules were found throughout the lungs and dorsal body wall. Morphological analysis reveals the fungus grows and sporulates at 25 C, but is unable to grow at 37 C. It produces arthroconidia and their morphology will be detailed. Bayesian analyses of seven loci confirm placement in the Onygenales and indicate that the fungus is distinct from its closest known relatives (*Chrysosporium* spp. and *Coccidioides* spp.). Its genome has been sequenced by 454 shotgun sequencing and preliminary analysis of the 21 Mb sequence will be presented.

673. The role of the autophagic gene atg8 in the *Ustilago maydis* life cycle

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Autophagy is a complex degradative pathway in which cytosolic material including organelles are randomly sequestered within double membranous vesicles termed autophagosomes. These autphagosomes are subsequently targeted to the lytic compartment where their contents are degraded by vacuolar hydrolases and the components recycled. The pathway was initially described as a cellular response to nutrient starvation and as the primary way to recycling obsolete proteins. In recent years, extensive screening, mostly in *S. cerevisiae,* has led to the identification of the genetic components driving autophagy, the ATG genes. Autophagy is now implicated in a rather varied collection of developmental processes in many other organisms.

In Aspergillus oryzae, deletion of the ATG8 ortholog leads to a defect in conidiation and conidial germination. In the plant pathogenic fungus *Magnaporthe grisea* deletion of the ATG8 ortholog, resulted in loss of autophagy induction, proper appresorium development and pathogenicity. Likewise, deletion other autophagic genes (Mgatg2, Mgatg4, Mgatg5, Mgatg8, Mgatg9, Mgatg18 orthologs) in the same fungus produced strains that exhibit defects in autophagy, reduction in conidiation, spore germination, appresorium turgor and lost of pathogenicity.

Ustilago maydis is a basidiomycete plant pathogenic fungus responsible for corn smut disease of maize and an important model organism. We have initiated inquiries to address potential roles of autophagy during *U. maydis* saprobic and pathogenic development. We deleted the ATG8 ortholog in *U. maydis* and showed that the gene is required for proper budding morphology as is evident by the increased number of lateral buds in mutant strains. Additional, umatg8 deletion affects the ability of the strains to survive during carbon starvation. The mutant strains also exhibit a substantial decrease in virulence accompanied by a reduction in gall formation and spore production.

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