

Saturday 31 March

## Parallel session 2: Sex and Sexual Development

### PS2.1

#### **A mating loci in *Coprinopsis cinerea* differ in numbers of homeodomain transcription factor genes**

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The 25 kb-long *A* mating type locus in the mushroom *Coprinopsis cinerea* controls defined steps in the formation of a dikaryotic mycelium after mating of two compatible monokaryons as well as the formation of the fruiting bodies on the established dikaryon. Usually, three paralogous pairs of divergently transcribed genes for two distinct types of homeodomain transcription factors (termed HD1 and HD2 after) are found in the *A* locus. For dikaryon formation and regulation of sexual development, heterodimerization of HD1 and HD2 proteins from allelic pairs from different *A* loci is required. In some *A* loci found in the wild, alleles of gene pairs are not complete or one of two genes have been made in-active. Functional redundancy allows the system still to work as long as an *HD1* gene in one and an *HD2* gene in the other allelic gene pair are operative. In this study, we present for the first time two completely sequenced *A* mating loci of *C. cinerea*. Evidences for gene duplications, deletions and inactivations are found. The loci differ in the number of potential gene pairs (five versus three), in genes that have been lost in evolution and in genes that are still present but have been made inactive.

### PS2.2

#### **Systematic deletion of homeogenes in an ascomycete fungus supports analogy of clamp and crozier in Dikaryomycota**

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The dikaryotic state, a unique characteristic shared by superior fungi, is maintained by similar structures, the clamp in Basidiomycetes and the crozier in Ascomycetes. A debate is going on regarding whether these structures are analogous or homologous. The essential role of basidiomycetes homeodomain transcription factors (HDTF) in clamp formation led us to examine their possible involvement in crozier formation in Ascomycetes. We identified the seven homeobox genes of the ascomycete filamentous fungus *Podospora anserina* and constructed mutants inactivated for either one or several homeogenes. Croziers developed normally in these mutants, including those inactivated for up to six homeogenes. However, two mutants are defective in the beak formation, an essential differentiation for efficient ascospore ejection. This defect can be rescued by wild-type maternal hyphae, thus demonstrating its maternal origin. Multiple mutant analyses revealed interactions between homeobox genes, suggesting that they operate as a complex network. Like in animals and plants, HDTF are involved in shaping multicellular structures in Ascomycetes. In contrast, HDTF are not required for the dikaryotic tissue formation and development. These data demonstrate that the crozier of Ascomycetes and the clamp of Basidiomycetes are under different genetic control, suggesting that they are analogous and not homologous structures.

### PS2.3

#### **Sexual Development and Spore Pathogenesis of *Cryptococcus***

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*Cryptococcus neoformans* is a human pathogen that causes fungal meningoencephalitis, primarily in immunocompromised individuals, resulting in over one million cases of cryptococcosis and ~600,000 deaths annually worldwide. Infection appears to occur via a respiratory route from environmental sources such as disrupted soil and bird droppings. Although the vegetatively growing yeast form of *C. neoformans* has been shown to cause disease in animal models, the infectious particles in human disease are not known. Given their small size and resistance properties, spores are a likely cause of natural infections.

Spores are the products of sexual development of *C. neoformans*, which appears to occur in the environment and can also be induced in the laboratory. To understand molecular events governing sexual development, we carried out an analysis of gene expression over time during development using microarray analysis. We are using these data to identify genes and regulatory elements involved in spore formation.

To investigate whether/how spores may function as infectious particles, we purified spores to homogeneity and evaluated them. Using a variety of methods, we determined that spores are covered by a thick coat that is both morphologically and compositionally distinct from yeast cells that appears to provide spore resistance to environmental stress. We are continuing to explore the basic physical and biochemical properties of spores with a focus on the spore surface.

To understand how spores interact with the host immune response, we carried out virulence assays using a mouse intranasal infection model of cryptococcosis. We discovered that spores are disease-causing particles; mice infected with spores developed disseminated cryptococcosis. Strikingly, however, we found that spore-mediated disease is distinct from yeast-mediated disease. Spores led to higher burdens of *C. neoformans* in the brain, and the mice were more likely to show signs of central nervous system (CNS) disease than yeast-infected mice. Furthermore, yeast strains that are avirulent in mice can cause fatal CNS disease when infected as spores, revealing that disease outcomes can differ depending on the infectious particle to which the host is exposed.

These data represent the first glimpses into the process of spore biogenesis, the basic properties spores, and the host response to these novel particles. Continuing studies of spores promise to reveal how *C. neoformans* infects the host and ultimately causes disease.

#### PS2.4

##### **Living together. Genetics and genomics of the dikaryotic lifestyle**

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In basidiomycetes, karyogamy occurs immediately before the meiosis and long after plasmogamy. Because of this delay, these fungi spend most of their life cycle as dikaryotic mycelia containing two separate nuclei per cell. We are studying the genetic and genomic mechanisms of dikaryosis as a model for internuclear communication and its impact on gene expression. To tackle it, we have sequenced the genomes of the two nuclei present in the strictly dikaryotic basidiomycete *Pleurotus ostreatus* in collaboration with the JGI, and we have used RNAseq to monitor the differences in gene expression in monokaryotic and dikaryotic mycelia. The questions dealt with the genome synteny, the genetic control of the monokaryotic/dikaryotic lifestyle, the differential expression of genes and alleles in the two conditions, and the identification of genes specifically expressed in monokaryons and dikaryons.

The synteny analysis revealed multiple single and nested chromosomal inversions that suppress chromosomal crossover and produce supergenes that can be associated to quantitative trait loci in some cases. Moreover, synteny studies of different fungi suggested that inversions can be a main evolutive mechanism in basidiomycetes.

The sequence analysis revealed the presence of genome-specific genes and of several helitron families involved the promoting structural heterozygosis and the amplification of genes involved in degradation processes.

The RNAseq experiments identified a number of genes whose expression is tightly associated to the monokaryotic/dikaryotic condition. Some of them were known to be involved in the maintenance of the cellular conditions, but others could be responsible for the differences between the two lifestyles.

#### PS2.5

##### **The transcription of the putative mating type genes *sexM* and *sexP* is regulated by trisporic acid in *Mucor mucedo***

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The putative mating type locus of mucoralean fungi consists of a single HMG-domain transcription factor gene, *sexM* or *sexP*, flanked by genes for a RNA helicase and a triose phosphate transporter. Sequencing of the sex locus of *Mucor mucedo* follows this general scheme as previously found in *Phycomyces blakesleeanus*, *Rhizopus oryzae* and *Mucor circinelloides*. Only the TP transporter is deleted in both mating types of *Mucor mucedo*. The participation of transcription factors in the regulation of sexual development in mucoralean fungi has not been proven yet. We performed transcriptional analysis of *sexM* and *sexP* by quantitative real-time PCR and found that under sexual stimulation, i.e. either in cross cultures or after addition of the mating pheromone trisporic acid, both transcripts are upregulated. Interestingly, the promoter sequences controlling *sexM* and *sexP* show no similarities to each other. Comparison of the promoter sequences of *sexP* from different zygomycetes show a conserved transcription factor binding site, whereas the promoter sequences of *sexM* of different zygomycetes do not show any conserved regions. At the protein level, both transcription factors are completely unrelated with each other, and only *sexM* harbors a putative nuclear localization sequence. Heterologous expression of *sexM* coupled to GFP in *Saccharomyces cerevisiae* shows that *sexM* is indeed accumulated in the nucleus of yeast.

## PS2.6

### Interspecific sex in grass smuts and the genetic diversity of their pheromone-receptor system

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The grass smuts comprise a speciose group of biotrophic plant parasites, so-called Ustilaginaceae, which are specifically adapted to hosts of sweet grasses, the Poaceae family. Mating takes a central role in their life cycle as it initiates parasitism by a morphological and physiological transition from saprobic yeast cells to pathogenic filaments. As in other fungi sexual identity is determined by specific genomic regions encoding allelic variants of a pheromone-receptor (PR) system and heterodimerising transcription factors. Both operate in a biphasic mating process that starts with PR-triggered recognition, directed growth of conjugation hyphae and plasmogamy of compatible mating partners. So far, studies on the PR system of grass smuts revealed diverse interspecific compatibility and mating type determination. However, many questions concerning the specificity and evolutionary origin of the PR system remain unanswered. Combining comparative genetics and biological approaches we report on the specificity of the PR system and its genetic diversity in 10 species spanning about 100 million years of mating type evolution. We show that three highly syntenic PR alleles are prevalent among members of the Ustilaginaceae favouring a triallelic determination as the plesiomorphic characteristic of this group. Furthermore, interspecies sex tests detected a high potential for hybridisation that is directly linked to pheromone signalling as known from intraspecies sex. Although the PR system seems to be optimised for intraspecific compatibility, the observed functional plasticity of the PR system increases the potential for interspecific sex, which might allow the hybrid-based genesis of newly combined host specificities.

## PS2.7

### The pheromone system of *Hypocrea jecorina* (*Trichoderma reesei*) and its regulation by photoreceptors upon sexual development

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Discovery of sexual development in the biotechnologically important ascomycete *Trichoderma reesei* (*Hypocrea jecorina*) as well as detection of a novel class of peptide pheromone precursors in this fungus indicates promising insights into its physiology and lifestyle. We investigated the *H. jecorina* pheromone-system and its regulation by the light response pathway.

The pheromone receptors HPR1 and HPR2 confer female fertility in their cognate mating types. Neither pheromone precursor genes nor pheromone receptor genes of *H. jecorina* were transcribed in a strictly mating type dependent manner, but showed enhanced expression levels in the cognate mating type. Transcripts of the pheromone precursor genes respond to the presence of a mating partner. In the female sterile *T. reesei* strain QM6a, these responses were altered. Inappropriate reaction to the mating partner may hence cause female sterility in QM6a.

In agreement with the dependence of sexual development on light, the photoreceptors BLR1 and BLR2 were found to impact fruiting body formation, although they are not essential for mating. In contrast, ENV1 was needed for female fertility. BLR1, BLR2 and ENV1 negatively regulate transcription of both pheromone receptors as well as peptide pheromone precursors in a mating type dependent manner. Thereby, the effect of ENV1 is most intense and at least partially mediated via regulation of *mat1-2-1*. ENV1 is concluded to be crucial for balanced regulation of genes regulated in a mating type dependent manner by the MAT-locus, and consequently for determination of sexual identity and fruiting body formation.

## PS2.8

### **A developmental protein as scaffold for a MAP kinase pathway controlling fungal fruiting body development?**

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The formation of fruiting bodies during sexual development in filamentous fungi is very complex and involves the generation of new cell types. Using the homothallic ascomycete *Sordaria macrospora* as a model system, we have identified a number of developmental proteins essential for this differentiation process. One of these, PRO40, the homolog of *Neurospora crassa* Soft, was employed for protein-protein interaction studies. In a yeast two-hybrid screen, the MAP kinase kinase (MAPKK) MEK1 was identified as a putative PRO40 interaction partner, and further analysis revealed an additional interaction between PRO40 and the MAPKKK MIK1. Both kinases can be assigned to the cell wall integrity pathway by their homology to the corresponding yeast kinases. Interestingly, while a *pro40* knockout strain is impaired in sexual development and hyphal fusion, a *mek1* knockout strain shows additional defects in vegetative growth and resistance to cell wall stress. We hypothesize that PRO40 scaffolds the MAPKKK and the MAPKK of the designated cell wall integrity pathway of *S. macrospora* in a development-specific manner leading to the formation of fruiting bodies.

## Saturday 31 March

### Parallel session 3: Genomes and Genome Evolution

## PS3.1

### **Evolution of Obligate Parasitism in the White Rust Pathogen of *Arabidopsis thaliana***

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Biotroph evolution and molecular mechanisms of biotrophy in eukaryotic plant pathogens are poorly understood. To address evolutionary and mechanistic aspects of biotrophy we sequenced the genome of the obligate biotroph oomycete *Albugo laibachii*. Comparisons with other oomycete plant pathogens revealed independent loss of biosynthetic pathways while other proteins like HSP90 show an unexpected expansion. Biotrophic organisms require “effectors” to suppress host defence; we revealed RXLR and Crinkler effectors shared with other oomycetes, and furthermore discovered a novel class of effectors, the “CHXCs”.

A requirement for a biotrophic life style is an intimate complex haustorial interface with parasitized cells. Beside its role in nutrient uptake, little is known about structural features, protein content and protein trafficking. We used proteomics and cytological approaches to dissect the haustorial interface and revealed the accumulation of a HSP90 subclass within the extrahaustorial matrix.

We hypothesize that evolution of biotrophy involves a series of steps: step 1, involving progressively more effectors to suppress defence that might evolve from conserved proteins step 2, attenuated activation of defence by reduction in the inventory of cell wall hydrolyzing enzymes, resulting in, step 3, weak selection to maintain certain biosynthetic pathways if the products of the pathways can be obtained from the host. This results in progressively more comprehensive auxotrophy and culminates in irreversible biotrophy.