XI
FUNGAL EVOLUTION AND SPECIATION

Chair:

Gillian Turgeon

& Irina S. Druzhinina

XIo-1

Mating type, signal transduction and the evolution of self-fertility in *Aspergillus nidulans*

Paul S Dyer*, Mathieu Paoletti, Fabian A Seymour, David B Archer

School of Biology, Universityof Nottingham, Nottingham NG7 2RD, UK

Sexual reproduction by self-fertilization (selfing) is particularly common in flowering plants and fungi and is thought to confer certain ecological benefits. Although much is understood about the genetic controls and signalling pathways involved with out-crossing in plants and fungi, surprisingly little is known about the genetics and cellular basis of self-fertilization. We investigated the genetic basis of selfing in the homothallic fungus *Aspergillus nidulans* (teleomorph *Emericella nidulans*). We previously demonstrated that alpha-domain and high-mobility group (HMG) mating-type (MAT) genes, found in outcrossing species, are present in the same genome of *A. nidulans*. We now present results from RACE-PCR, used to assess gene expression, and from over-expression experiments of the alpha-domain and HMG mating-type genes (termed *MAT1* and *MAT2* respectively), used to assess effects on vegetative growth and sexual differentiation. Antisense RNA manipulation was also used to determine whether MAT gene activity is essential for sexual development. Genome analysis revealed the presence of a series of genes for a pheromone-response MAP-kinase signalling pathway characteristic of heterothallic sexual species. Sexual reproduction was found to be correlated with significantly increased expression of MAT genes and representative genes of the pheromone-response pathway. These results indicate that selfing in *A. nidulans* involves activation of the same cellular mating pathways characteristic of sex in outcrossing species, i.e. self-fertilization does not by-pass the normal requirements for sexual signalling between partners, but instead requires activation of these pathways within a single individual. However, unlike heterothallic species pheromone signalling appeared to be independent of MAT gene control. Comparison of the MAT loci of *A. nidulans* to other Aspergilli revealed a degree of microsynteny and suggested a model for the evolution of homothallism in this species.

Evolutionary origins of aflatoxin, sterigmatocystin and fumonisin gene clusters

Nora Khaldi*, Kenneth H. Wolfe

Smurfit Institute of Genetics. University of Dublin Trinity College Dublin 2 Ireland

Fungal toxins have significant affects on human, animal and plant health. In many species, the genes required for toxin biosynthesis are known to be clustered in the genome. So far little work has targeted evolutionary perspectives: How, when and why did these clusters assemble? Were they formed by horizontal gene transfer? Do the genes evolve in a similar way to other genes in the genome? We examined these questions using the aflatoxin (sterigmatocystin) cluster present in some *Aspergillus* species, and the fumonisin cluster present in *Gibberella moniliformis*. We show using phylogenetic analysis that orthologs of the fumonisin and aflatoxin genes are present in the genomes of a number of euascomycete fungi that do not produce the toxins. This suggests that the genes involved in the toxin biosynthesis pathways were present in the common ancestor of Pezizomycotina, and have not been horizontally transferred. In the same manner we used phylogenetic trees to find orthologs of the genes flanking the fumonisin cluster in G. *moniliformis*. This revealed that the flanking genes are syntenically conserved and adjacent in F. *graminearum*, indicating that the cluster assembled by insertion. These results also suggest that clustering of the genes was selected for, and that the formation of a cluster correlates with the toxin production. We also show for both clusters that their genes underwent acceleration of evolutionary rate after the cluster was formed. Finally, we discuss possible ancestral functions of the genes in the clusters.

XIo-3

How can the reconstruction of GMC-flavoprotein phylogeny contribute to our understanding of fungal evolution?

Marcel Zamocky*, Clemens Peterbauer, Roland Ludwig, Dietmar Haltrich

Research Centre Applied Biocatalysis, Petersgasse 14, A-8010 Graz, Austria

We reconstructed the phylogeny of GMC oxidoreductases - a widespread group of flavoenzymes present in all kingdoms of life [1]. In the sequenced fungal genomes GMC superfamily can be traced in several evolutionary lines. A representative set of selected protein sequences of this superfamily, chosen mainly from fungal genomes, was aligned with Clustal X. Catalytically and structurally important residues were found to be conserved among all analysed open reading frames. Subsequently, the phylogeny was reconstructed with neighbour-joining and maximum-likelihood methods. We demonstrated the abundant occurrence of two important GMC evolutionary lines typical for fungi: cellobiose dehydrogenases (CDH) and pyranose 2-oxidases (P2O). Cellobiose dehydrogenases are segregated in two distinctive classes that are typical for basidiomycete or ascomycete producers. A third-divergent clade of parasitic ascomycete CDH exists with yet unknown function. Among pyranose 2-oxidases three distinct clades exist. The well known P2O representatives come all from closely related white rot fungi but a putative ascomycete P2O clade is not investigated at the protein level yet. We will compare the reconstructed tree of this superfamily with evolutionary relationship of the corresponding fungi based on the analysis of 18S-rDNA [2] and discuss the discrepancy. Analysis of the phylogeny of enzymes involved in lignocellulose degradation can thus contribute significantly to our understanding of the evolution of fungal parasitism.

References

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Fungal class II peroxidases: phylogenetic divergence for specific functions?

Taina K Lundell*, Kristiina S Hildén, Miia R Mäkelä, Annele I Hatakka

Department of Applied Chemistry and Microbiology, Division of Microbiology, University of Helsinki, Finland

Class II secretory heme peroxidases such as the lignin-degrading peroxidases (LPs) of basidiomycetous fungi show diverse functions as biocatalysts (lignin breakdown, conversion of xenobiotics, bleaching of coloured compounds and polymeric dyes) but minor differences in protein structure. According to the primary structure, the lignin, manganese and versatile peroxidases (LIPs, MNPs and VPs) may be divided into several gene subfamilies that overlap between basidiomycetous taxons. However, within one gene family the number of introns and gene splicing bring about several different gene organisations, which is more dependent on the fungal species than the LP coding sequence. In the genome of the lignin-degrading, white rot model basidiomycete, Phanerochaete chrysosporium, 10 lip, 5 mnp and one additional heme peroxidase encoding gene are found. On the contrary, only a few LPs have been identified so far when the fungus grows on lignocellulose. We have studied another well-lignin degrading and naturally wood-colonising saphrophytic white rot fungus, Phlebia radiata. This basidiomycete expresses a versatile set of extracellular enzymes including two divergent MNPs, three LIPs and one to two laccases upon growth on solid or milled wood. Molecular evolutionary sequence analysis of the LPs reveal clustering of the P. radiata lip genes but significant divergence with the two mnp genes, one short and the other long, thereby supporting three main evolutionary families within the class II fungal heme peroxidases. However, gene organisation supports more recent duplication of the short lip-vp-mnp type genes irrespective of fungal taxon, separated away from the cluster of the typical long fungal mnp genes and the Coprinus-peroxidase branch. Interestingly, evolutionary grouping coincides with the function and catalytic activity of the fungal class II peroxidases. Structure-function relationship of the LPs is also discussed based on in vitro reactions and differential expression upon degradation and growth on wood.

XIo-5

Dynamics of dsRNA mycoviruses in black *Aspergillus* populations

Anne D. van Diepeningen*, Fons J.M. Debets, Rolf F. Hoekstra

Laboratory of Genetics, Plant Sciences, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, the Netherlands

Approximately ten percent of all examined 668 representatives of black *Aspergillus* species, independent of worldwide location, were infected with double-stranded RNA mycoviruses. These isometric viruses (25-40 nm diameter) contained a variety of often multiple segments of different dsRNA sizes ranging from 0.8 to 4.4 kb in size. In one strain the virus shows clear visible effects on its host with non-sporulating sectors. We quantified the fitness costs of these and more 'cryptic' virus infections on mycelial growth rate and spore production, and on competitive ability with respect to other strains under different growth conditions. Mycovirus infection proved detrimental in all these measures. The reduced success in interference competition due to mycovirus infection belies coevolution of mycovirus and host to a mutually beneficial symbiosis like in killer virus systems in yeast and smut and agrees more to recent infections. For a stable virus infection frequency in the black *Aspergillus* population, fitness costs and spontaneous loss should be balanced with new infections. Implications of even small viral fitness effects combined with the observed transmission limits for host and mycovirus are discussed.