I

PLENARY LECTURES

Io-1

Focus on cell wall assembly.... a challenging final step on the road from genes to the understanding of fungal morphogenesis

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In its many morphological expressions, and with few exceptions, the cell wall is the ultimate manifestation of fungal differentiation. Much of what is remarkable in a fungus can be ascribed directly to its cell wall. The success of fungi in the ecological, phylogenetic, morphogenetic, or pathogenic arenas can be largely attributed to their ability to elaborate suitable cell walls endowed with the necessary physical and chemical properties for the task at hand. Understanding the genesis of a complex microfibrillar cell wall requires elucidation of the 3-D nature of the secretion process and detailed knowledge of the biochemistry and biophysics of polymer assembly at the cell surface. Mere excretion of wall materials is not likely to produce either a morphologically-normal or a fully-functional cell wall. Whereas conventional biochemistry plus molecular genetics have generated an impressive catalog of the many proteins that constitute, catalyze or control the cell wall and its biogenesis, deciphering the orderly assembly of the cell wall remains a formidable challenge. The seemingly simple act of extending the cell surface requires not only spatially coordinated synthesis of new components but also exquisite control to open up the wall fabric in a precise amount to allow insertion of new components without risking cell integrity. Earlier findings demonstrating that chitin microfibrils can be assembled *in vitro,* and identifying specific microvesicles (chitosomes) as dedicated carriers of chitin synthetase zymogen, advanced our knowledge of microfibril biogenesis but left unanswered key aspects of the 3-D secretion process and crucial details of the spatial integration of the microfibrils into the wall fabric. Currently, by taking advantage of fluorescent molecular tags that, surprisingly, do not seem to interfere with normal cell physiology, we have a powerful and spectacular tool to follow the spatial behavior and dynamics of specific molecules and organelles inside the growing fungal cell. Valuable insight has been gained on the dynamics of the microtubular cytoskeleton (see local abstract by Mouriño-Pérez *et al.*) and the polarized displacement of chitin synthetase (CHS3 & CHS6) from distal regions of the hyphal tube to the Spitzenkörper at the growing tip (see local abstract by Riquelme *et al.*). These and other experimental findings represent starting steps to construct and/or validate a 3-D model of hyphal morphogenesis based on the mathemaically-derived VSC (vesicle supply center) concept.

Transcriptional profiling and regulatory mechanisms associated with conidial germination and colony development in *Neurospora*

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We have constructed a 70 base oligomer microarray to 10,526 genes predicted genes in the genome of the filamentous fungus, *Neurospora crassa*. As a proof of principle, we estimated the relative gene expression levels and changes in gene expression during conidial germination. Remarkable consistency of mRNA profiles with previously published northern data was observed. Genes were hierarchically clustered into groups with respect to their expression profiles over the time course of conidial germination and a functional classification database (FunCat) was employed to characterize the global picture of gene expression. Our transcriptional profiling data correlate well with biochemical and physiological processes associated with conidial germination.

The *N. crassa* genome has at least 206 predicted transcription factor genes. Strains containing mutations in almost all of the predicted 206 predicted transcription factor genes have been constructed (K. Borkovich and J. Dunlap, personal communication). To define the transcriptional regulatory network of *N. crassa*, we are profiling these transcription factor mutants during vegetative growth, as compared to a wild-type *N. crassa* strain. Motif searches among putative target genes have identified a number of consensus sequences that may represent novel cis-acting regulatory elements for particular transcription factors, the vast majority of which have unknown biological roles.

Io-3

Mechanisms of genome-wide regulation of secondary metabolite gene cluster expression

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Natural product (i.e. secondary metabolite) formation is commonly associated with sporulation processes in fungi; a coupling known to be connected through shared signal transduction pathways. One target of the protein kinase A signaling pathway, LaeA, was recently reported as a global regulator of secondary metabolite gene clusters in *Aspergillus spp*. and is a virulence factor in the pathogens *A. fumigatus* and *A. flavus.* Here we present several lines of evidence demonstrating that LaeA regulation of gene clusters is chromatin location specific. Genomic profiling of both null and over expression *laeA* mutants has uncovered over a dozen LaeA regulated clusters, a high proportion of them located to sub-telomeric regions of the chromosome. Detailed studies of the *A. nidulans* (ST) gene cluster demonstrates that removal of a gene from the cluster relieves it from LaeA regulation; the opposite is seen when a non-cluster gene is placed within the ST cluster. Furthermore, deletion of histone modifying proteins partially remediate the *laeA*Δ phenotype. We present a model of chromatin mediated regulation of secondary metabolite gene clusters by LaeA.

Fruiting body development in ascomycetes: pro(s) and their mat(es) at work

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During sexual development, most mycelial ascomycetes form typical fruiting bodies called ascomata or ascocarps. These are highly complex, multicellular structures with many fruiting body forming tissues that surround the asci with their meiospores (ascospores). While cells of the fruiting body arise from haploid hyphae, the asci originate from dikaryotic hyphae. In recent years, we have investigated fruiting body development in the homothallic fungus *Sordaria macrospora*, which is closely related to the heterothallic pyrenomycete *Neurospora crassa*. Although homothallic, *S. macrospora* carries a mating (**mat**) type locus [1], resembling a hybrid of the two mating type loci from A and a strains from *N. crassa*. Knock-out strains of the mat locus encoded transcription factor Smta1 and cross-species microarray hybridizations unequivocally demonstrate that the mat locus regulates genes of the pheromone signalling pathway and is essential for fruiting body development in a homothallic fungus [2]. The sum of our experimental data indicate that conserved signalling molecules are directly involved in multicellular development [3]. However, components acting further downstream are yet unknown. Using a collection of **pro** mutants, developing only immature fruiting bodies, we have isolated and characterized components that are directly involved in the formation of ascocarps [4]. One class of mutants shows defects in lipid or amino acid metabolism [5] [6], while another one carries mutated developmental genes encoding proteins that are conserved from fungi to mammals [7]. Currently, it can be speculated that the identified proteins are components of a yet unknown signal transduction pathway controlling differentiation processes during fruiting body formation.

[1] Pöggeler S, *et al.* (1997) Genetics 147: 567-580
[2] Nowrousian M, *et al.* (2005) Mol Gen Genomics 273: 137-149
[3] Mayrhofer S, *et al.* (2006) Genetics (in the press)
[4] Masloff S, *et al.* (1999) Genetics 152: 191-199;
[5] Nowrousian M, *et al.* (1999) Mol Cell Biol 19: 450-460
[6] Kück U (2005). Mol Gen Genomics 274: 307-315
[7] Pöggeler S & Kück U (2004) Eukaryotic Cell 3: 232-240

Io-5

Analysis of polarized growth in *Aspergillus nidulans*

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Polarized growth is the mechanism by which filamentous fungi extend their hyphae. Microtubules (MT) and filamentous actin (F-actin), in combination with their corresponding motor proteins, kinesins, dynein and myosins, play crucial roles in this process. The exact contribution of the MT cytoskeleton, however, is still under debate. Genetic, biochemical and cell biological approaches in *A. nidulans* and other fungi led to a modified view of many aspects within the past few years. There is increasing evidence that MT strings, which are visualized by immunostaining or GFP-tagging, consist of several MTs and their dynamics appears to be different in fast-growing hyphal tips as compared with young germlings. Whereas the spindle pole bodies were considered as the only or the main microtubule organizing centres (MTOCs) in filamentous fungi, it appears that several additional MTOCs are responsible for the generation of the MT array. In addition to new insights into the MT network and its dynamics, the roles of several kinesins have been elucidated recently and their interplay with dynein investigated. It became clear that MT functions are interwoven with those of the actin cytoskeleton and that three main structures are required for polarized growth, the Spitzenkörper (vesicle supply centre), the polarisome and probably cell end markers at the cortex. We propose a model for polarized growth, where the actin cytoskeleton and the polarisome are crucial for hyphal extension and the MT cytoskeleton continuously provides the building material within vesicles to the Spitzenkörper and determines growth directionality by delivery of cell end marker proteins.

- Horio, T., and Oakley, B.R. (2005) The role of microtubules in rapid hyphal tip growth of *Aspergillus nidulans*. *Mol. Biol. Cell* 16, 918-926.
- Konzack, S., Rischitor, P., Enke, C. & Fischer, R. (2005). The role of the kinesin motor KipA in microtubule organization and polarized growth of *Aspergillus nidulans*. *Mol. Biol. Cell* 16, 497-506.
- Requena, N., Alberti-Segui, C., Winzenburg, E., Horn, C., Schliwa, M., Philippsen, P., Liese, R. & Fischer, R. (2001). Genetic evidence for a microtubule-destabilizing effect of conventional kinesin and analysis of its consequences for the control of nuclear distribution in *Aspergillus nidulans*. *Mol. Microbiol.* 42, 121-132.
- Rischitor, P., Konzack, S. & Fischer, R. (2004). The Kip3-like kinesin KipB moves along microtubules and determines spindle position during synchronized mitoses in *Aspergillus nidulans* hyphae. *Euk. Cell* 3, 632-645.
- Veith, D., Scherr, N., Efimov, V. P. & Fischer, R. (2005). Role of the spindle-pole body protein ApsB and the cortex protein ApsA in microtubule organization and nuclear migration in *Aspergillus nidulans*. *J. Cell Sci.* 118, 3705-3716.

Iron metabolism in *Aspergillus* and its relation to virulence

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Iron is an essential element for all eukaryotes because it serves as a cofactor in key metabolic processes such as energy production and nucleotide biosynthesis. As this metal is required by both, the pathogen and the host, its control is a central battlefield deciding about the fate of an infection. Accordingly, mammalians and pathogens have evoked sophisticated strategies to either acquire and/or hide iron. *Aspergillus fumigatus* is a saprophytic fungus present in almost all decaying organic materials. At the same time it is the most common air-borne fungal pathogen of humans. Clinical manifestations range from allergic reactions to invasive disease (aspergillosis), in particular in immunocompromised patients. We have shown that *A. fumigatus* lacks specific uptake systems for host iron compounds heme, ferritin and transferrin. However, it employs three, partially redundant, iron uptake mechanisms: (i) low-affinity ferrous iron uptake, (ii) reductive iron assimilation, and (iii) siderophore-mediated iron uptake. Siderophores are low-molecular-weight, iron-specific chelators, which are excreted to mobilize extracellular iron. Furthermore, specific intracellular siderophores are used to store iron and to prevent oxidative stress. Siderophore biosynthesis and uptake is repressed by iron and this regulation is mediated by the GATA-type transcription factor SreA. Comparing *A. fumigatus* wild type and a SreA-deficient mutant by microarray-based genome-wide expression profiling revealed new components of the fungal iron metabolism, down-regulation of respiratory functions by iron starvation, and upregulation of antioxidative enzymes in the SreA-deficient mutant. Together, these results expand our understanding of global iron regulation and its link to virulence of *A. fumigatus*.

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Io-7

Monosaccharide catabolic pathways controlling extracellular enzyme induction in *Hypocrea jecorina*

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The pantropical ascomycete *Hypocrea jecorina* (anamorph: *Trichoderma reesei*) is known to secrete an extracellular enzyme system including cellulases, hemicellulases and pectinases capable of degrading plant cell wall polymers The corresponding breakdown products are taken up and catabolized within different pathways, whose activity in turn regulates the transcription of these extracellular enzymes. While d-glucose is degraded via the ubiquitous glycolytic pathway, fungal-specific pathways have evolved for the catabolism of the other hemicellulose monosaccharides such as the pentoses D-xylose and L-arabinose and the hexose D-galactose. I will review the components of these pathways and their regulation and implication on extracellular enzyme production. Particular emphasis will be given on a novel pathway for D-galactose catabolism which makes use of various enzymes of the pentose catabolic pathways. The latter is of industrial interest because D-galactose is part of the disaccharide lactose (1,4-O-β-D-galactopyranosyl-d-glucose) a potent inducer of cellulases.

Systematic analysis of SNARE molecules in *Aspergillus oryzae*

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Secretion of large amounts of enzymes is a key abilityfor the industrial use of filamentous fungi. However, our understanding of the intracellular protein transport in filamentous fungi is still in its initial stages. To facilitate an in depth understanding of global vesicular trafficking in *Aspergillus oryzae*, we focused on SNAREs (soluble NSF attachment protein receptors) that have a central role in the fusion of transport vesicles with target organelles. We identified 21 genes that encode putative SNARE proteins from the *A. oryzae* genome database. All of these genes excepting for *Aovam3* (1) encoding the vacuole-localized homolog of *VAM3* in *Saccharomyces cerevisiae* were subsequently cloned and expressed as enhanced green fluorescent fusion proteins in *A.oryzae*. Fluorescence microscopy revealed a predominant localization of the AoUfe1, AoSec20, AoUse1, and AoSec22 (orthologs of ER-resident SNAREs in *S. cerevisiae*) at the ER. While those of the Golgi-resident SNAREs, AoSed5, AoBos1, AoGos1, AoBet1, AoSft1, and AoVti1 localized at putative Golgi apparatus near the hyphal tips, the orthologs of plasma membrane SNAREs, AoSso1, AoSso2, and AoSnc1, and those of vacuolar SNAREs, AoVam7, AoNyv1, and AoVti1 in general showed similar intracellular distribution compared to their counterparts in *S. cerevisiae*. Orthologs of endosomal SNAREs, AoTlg1, AoTlg2, AoSyn8, AoVti1, and AoSnc1 localized to small punctate structures that were highly motile and resembled the recently identified putative endosomes (2), suggesting that they might be early endosome-like structures. Moreover, a few endosomal and vacuolar SNAREs, AoTlg1, AoVti1, AoNyv1, and AoSyn8 also appeared in putative late endosome/prevacuolar compartment described previously (1). While the plasma membrane resident SNAREs also localized at the septal membrane, the ER resident SNAREs appeared at two linear compartments divided by septum possibly reflecting peripheral ER. Strikingly, a few endosomal and vacuolar SNAREs also localized at the septa, suggesting that these SNAREs have certain specialized roles in filamentous fungi. These results suggest a conservation in the subcellular distribution of SNAREs between *A. oryzae* and *S. cerevisiae*. Interestingly, the endosomal and vacuolar SNAREs showed slightly distinguished and sometimes overlapped distribution. This systematic approach led us to some remarkable findings on vesicular trafficking in *A. oryzae*. For example, the overexpression of AoSed5 disrupted the cell polarity during conidial germination and hyphal growth. Further analysis suggested that excess AoSed5 altered the subcellular distribution of some organelles and the cytoskeleton possibly due to the defect in retrograde transport from the Golgi apparatus to the ER.

(1) Shoji *et al.*, Eukaryot. Cell, 5: 411-421 (2006)
(2) Higuchi *et al.*, BBRC,340:784-791 (2006)

Io-9

Peptide assembly line genes in fungal genomes: evolution and extinction of NRPS genes

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Nonribosomal systems are known to produce a variety of unique peptide structures. These systems of highly complex multidomain structures are only found in eubacteria and fungi, but absent in archae and higher eukaryotes, although most of their domains are universally present in different contexts. Fungal nonribosomal peptide synthetases (NRPS) have various properties not found in prokaryotes, which may help to explain this unexpected instability and loss. Fungal NRPS systems are generally fused entities of giant genes with sizes of up to almost 70 Kb, only scarcely interrupted by introns. Fungal genomes as certain bacterial ones may contain a surprisingly high amount of NRPS systems, for which mostly products and functions are unknown [1]. Genome wide sequencing efforts have provided information on the distribution of genes and gene clusters. Gene clusters like the ß-lactam system are highly conserved, and found in bacteria and ascomycetes. Fungal siderophores, however, are not found in bacteria, and the ferrichrome-type is detected in basidiomycetes and ascomycetes. Peptaibols, with up to 20 residues the most extended system, are restricted to certain ascomycetes. A variety of orphan NRPS-genes show varying architectures and have no matches. These genes are frequently degenerated to pseudogenes by frame shifts or mutations leading to stop codons. Their structural variability is significantly larger than in prokaryotic systems including distortions by insertions and deletions.

Here an analysis of fungal siderophore synthetases will be given. Contrary to the wealth of bacterial siderophores, fungi produce only a limited number of highly efficient peptides. The respective peptide synthetases show a surprising variety of domain organizations, indicating a complex evolutionary history [2]. Ferrichromes can be considered as perfect chelators, but their synthetases seem to evolve further by deletions and modifications. Such processes could be interpreted as streamlining to smaller interative systems.

A second type of assembly lines discussed are peptaibol synthetases. The *Trichoderma reesei* genome has provided information on two structural types of these unique peptides, which are produced in sets of structural analogs. The large variety of structural types leads to insights into the evolution of these systems. Starting from yet unknown prototypes new compounds are generated by combinatorial approaches in which the synthetase genes are altered by various processes.

Finally the current state of NRPS gene structure-function correlation will be reviewd.

[1] von Döhren, H. (2004) Biochemistry and general genetics of nonribosomal peptide synthetases in fungi. Adv. Biochem. Engin/Biotechnol 88, 217-264

[2] Schwecke, T., Göttling, K., Durek, P., Dueñas, I., Käufer, N.F., Zock-Emmenthal, N., Staub, E., Neuhof, T., Dieckmann, R., von Döhren, H. (2006) Nonribosomal Peptide Synthesis in *Schizosaccharomyces pombe* and the architecture of ferrichrome type siderophore synthetases in fungi, Chembiochem, 2006 Feb 27; [Epub ahead of print]

Estimating the geological ages of fungi: predictions from molecular phylogenetics and the fossil record

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Phylogenetic inference and fossil evidence are the most important ingredients in estimating ages of fungal lineages. Adding in the assumption that DNA substitutions accumulate following a molecular clock has the potential to extend available fossil evidence and date fungal divergences even where fossils are missing. Phylogenies for fungi are increasingly reliable, which should be increasing the accuracy of estimates of divergence times. However, the fungal fossil calibration points are few and difficult to interpret, and this combined with apparently chaotic evolutionary rate variation has resulted in an astonishing range of estimates for ages (e.g., for the first divergence in the Fungi, from 570 Ma Berbee and Taylor 1993 to 1,460 Ma in Heckman *et al.*, 2001). In this talk, we will review the fungal fossil record, explore the relationship between assumptions about identity of fungal fossils and estimates of lineage-specific rates of substitution, and speculate about the kinds of data that will improve estimates of fungal divergence dates in the near future.