

IVp-1

Cytology of conidial anastomosis tube induction, homing and fusion in *Neurospora crassa*

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Conidia of *Neurospora crassa* form conidial anastomosis tubes (CATs) which are morphologically and physiologically distinct from germ tubes, and under separate genetic control. The dynamic behaviour of nuclei, mitochondria and microtubules during macroconidial germination and fusion was analysed using a range of vital dyes and GFP labelling. We found that: (1) mitosis occurs much more rapidly in germ tubes (~ 15 min) than in ungerminated macroconidia (~ 3.5 h); (2) mitochondria are concentrated within germ tube and CAT tips; (3) both nuclei and mitochondria are physically connected to microtubules; (5) CAT fusion occurs after mitosis has been undergone in ungerminated macroconidia or germ tubes; (6) nuclei do not enter CATs prior to fusion; (7) the nuclei within germ tubes or CATs do not exhibit any special localization which can be associated with the pattern of CAT induction, homing or fusion; (8) microtubules extend through fused CATs from both conidial germlings which have fused with each other; (9) microtubules pass through fused CATs prior to mitochondria which are then followed by nuclei; and (10) organelle fluxes between fused conidial germlings are typically several orders of magnitude slower than those between fused hyphae in the mature colony.

Conidial anastomosis tubes in filamentous fungi

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In filamentous fungi a colony can arise from a single spore but it has been long appreciated that conidia and conidial germlings in close vicinity to each other commonly undergo fusion to produce an interconnected network of germlings. These germlings are connected by specialized hyphae called conidial anastomosis tubes (CATs). CATs have been described in 73 species of filamentous fungi covering 21 genera, and develop in nature and in culture. They have been shown to be morphologically and physiologically distinct from germ tubes in *Colletotrichum* and *Neurospora*, and under separate genetic control in *Neurospora*. CATs are short, thin, usually unbranched and arise from conidia or germ tubes. Their formation is conidium density dependent, and CATs grow towards each other. MAP kinase mutants of *Neurospora* are blocked in CAT induction. Nuclei pass through fused CATs and are potential agents of gene exchange between individuals of the same and different species. CAT fusion may also serve to improve the chances of colony establishment. In this presentation the defining characteristics of CATs are described, recent insights that have been gained into their cell biology are reviewed, possible roles of CATs are suggested, and key questions which need to be addressed about their biology are defined.

IVp-3

To fuse or not to fuse? Hyphal anastomosis in *Sordaria macrospora* mutants defective in fruiting body development

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During sexual development, the homothallic ascomycete *Sordaria macrospora* forms typical fruiting bodies (perithecia) which harbour asci containing eight ascospores. Starting from a single spore, the life cycle of *S. macrospora* can be completed within seven days in the laboratory. We use this ascomycete as a model system to study developmental genes controlling fruiting body development, and have already demonstrated that *S. macrospora* is a suitable experimental system for the discovery of components controlling cell differentiation. Using a forward genetic approach several mutants have been generated with defects in sexual development [1, 2]. Here we present the molecular genetic analysis of *pro22* and *pro40*, two sterile mutants showing an arrest after formation of immature fruiting bodies (protoperithecia) and being restricted in their ability to form perithecia. Using an indexed cosmid library [3] we were able to restore fertility in the two mutants by DNA-mediated transformation.

DNA sequence analyses demonstrate that the *pro22* ORF is interrupted by four introns and encodes a polypeptide of 1121 amino acids. Database searches show that PRO22 is an ortholog of Far11p from *Saccharomyces cerevisiae* [4] and HAM-2 from *Neurospora crassa* [5]. The *pro40* ORF is interrupted by a single intron and encodes a polypeptide of 1316 amino acids. Database searches revealed that PRO40 is an ortholog of SOFT from *N. crassa* [6].

Here we present evidence for two different cell fusion processes in *S. macrospora*. During sexual development, anastomoses are most probably prevented in both sterile mutants, while vegetative mycelia are still able to undergo hyphal fusion. Using differently labeled nuclei, the formation of homokaryotic and heterokaryotic mycelia was demonstrated by fluorescence microscopy. We conclude from our investigations that vegetative hyphal fusion is not a prerequisite for the formation of fruiting body maturation during the sexual cycle.

[1] Masloff, Pöggeler and Kück (1999) Genetics 152: 191-199

[2] Nowrousian, Masloff, Pöggeler and Kück (1999) Mol Cell Biol 1: 450-460

[3] Pöggeler, Nowrousian, Jacobsen and Kück (1997) J Microbiol Meth 29: 49-61

[4] Kemp and Sprague (2003) Mol Cell Biol 23: 1750-1763

[5] Xiang, Rasmussen and Glass (2002) Genetics 160: 169-180

[6] Fleißner, Sarkar, Jacobson, Roca, Read and Glass (2005) Euk Cell 4:920-930

Identification of genes involved in *Aspergillus nidulans*

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Filamentous fungi, such as *Aspergillus niger* and *Aspergillus oryzae* have been used secrete large amounts of native proteins for many industries. More recently the production of heterologous proteins has been attempted with limited success, as production yields obtained were often low. The link between the hyphal growth and protein secretion, primarily from the hyphal tip has been noted and several studies to improve protein secretion capacities through the production of hyperbranching mutants have been carried out.

A limited number of *Aspergillus* mutants are known to affect hyphal morphology, growth and polarity, including *cotA*, and *hbr* mutants. With the availability of genome sequences for the 3 species of *Aspergillus* and microarray technology, the identification of genes involved in morphological development can be established by transcriptome analysis of hyperbranching mutants and using bioinformatics to identify *Aspergillus* homologues of known genes.

The temperature sensitive *A. nidulans* mutant HbrB3 exhibits hyperseptation and shows a marked increase in hyphal branching at the restrictive temperature 40°C. RNA was isolated from the mutant strain HbrB3 and a wild type strain, R153, grown under continuous culture conditions at both 30°C and 40°C, and reverse transcribed with *cy3* and *cy5*. Analysis of microarrays probed with fluorescently labelled cDNA from these strains has been used to identify genes whose expression differs significantly in the mutant strain relative to the wild type. Functional analysis of genes using the *alcA* (alcohol dehydrogenase) conditional promoter is currently in progress. The ORF AN6796.2 when down-regulated show a hyperbranching phenotype, similar to that seen when *Ste20* is down-regulated. Initial investigation of AN5624.2 shows enlarged vacuoles when the gene is down regulated. A KU knockout strain is now available which will provide a quicker method of promoter exchange, allowing many genes to be functionally investigated.

IVp-5

Expressed genes during development of shiitake mushroom *Lentinula edodes* examined by serial analysis of gene expression

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Lentinula edodes (*L. edodes*) is a popular cultivated mushroom with high nutritional value and good taste. We used Serial Analysis of Gene Expression (SAGE) to analyze genes expressed in its developmental stages. SAGE is an efficient molecular method to count transcripts and identify novel genes, using 2 steps: 1) 9-15bp sequence tags extracted from mRNAs, and 2) Concatemers created from tags for sequencing. mRNAs from mycelium, primordium, fruiting body, and sporulating fruiting body were extracted to generate SAGE libraries. A total of 18 000 tags were collected from SAGE libraries: mycelium, 3350, primordium, 3400, fruiting body, 4500, and sporulating fruiting body, 7000 . About 440 unique tags were matched to the ESTs generated from primordial stage and catalogued by the Expressed Gene Anatomy Database (EGAD). We have also randomly sequenced more than 13 000 cDNAs for annotating more SAGE tags. Genes expressed at high levels in mycelium were generally expressed at low levels in primordium. Gene expression profiles from mycelium to primordium showed (1) initiation—stress response and specific signal transduction, (2) reconstruction of proteome—protein degradation, modification and biosynthesis, and (3) metabolic switch-over—altered biochemical pathways and structural components, especially hydrophobins. Genes highly expressed in sporulating fruiting bodies but not before sporulation revealed the genes playing important roles in sporulation. By comparing the SAGE libraries, we have a better understanding of *L. edodes* development. Results obtained from SAGE were validated by Northern and real-time PCR analyses of selected genes. Our studies indicate that SAGE is reliable to determine gene expression profiles and identify differentially expressed novel genes in mushroom development, providing data that are difficult to achieve by conventional methods.

Differential distribution of the endoplasmic reticulum network as visualized by the BipA-EGFP fusion protein in hyphal compartments across the septum of the filamentous fungus, *Aspergillus oryzae*

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The endoplasmic reticulum (ER), a universal organelle in eukaryotic cells, plays essential roles in the folding and modification of nascent secretory proteins and calcium homeostasis. In filamentous fungi the ER forms a tubular network in hyphae and can be visualized by the expression of the GFP (green fluorescent protein)-fused protein. In filamentous fungi the GFP-stained ER is known to show a gradient distribution from the apical region, suggesting that the ER supports the bulk flow of secretory proteins towards the hyphal tip and thus contributes to polar extension during filamentous growth. Although *in vivo* observation of the ER network has been performed in apical compartments, spatial difference of ER distribution in various regions of hyphae such as subapical compartments remains poorly understood. Subapical compartments are arrested in interphase while the apical compartment remains mitotically active. Contrarily to different cellular states in adjacent compartments, inter-compartment communication seems to be achieved via septal pores, which are plugged by the Woronin body to prevent excessive loss of the cytoplasm during hyphal lysis. However, spatial regulation of organelles in adjacent compartments across a septum has not been extensively investigated.

In this study we visualized the ER network by expression of the BipA-EGFP fusion protein in the filamentous fungus, *Aspergillus oryzae* and focused on the spatial difference of the ER distribution throughout hyphae. The ER formed an interconnected network with motility and displayed a gradient distribution from the apical region. The ER was also found as a tubular network along the septum, which was formed soon after the completion of septation. Interestingly, discontinuity of the ER network distribution was noticed between the adjacent compartments across the septum, suggesting that the cellular activities in these compartments were independently regulated although they are considered to communicate with each other through the septal pore. Moreover, the ER-visualized strain was subjected to a hypotonic shock, leading to hyphal tip bursting where the Woronin body plugs septal pores and prevents excessive loss of the cytoplasm. In the compartment adjacent to the burst apical tip, the ER network structure and motility were still retained. We also observed re-growth of hyphae from the plugged septa forming intrahyphal hyphae in which the ER network distribution, specialized for apical growth, was regenerated.

IVp-7

Comparative dynamics of the microtubular cytoskeleton in living hyphae of *Neurospora crassa* wild type, *ropy-1* and *ropy-3*

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By confocal and standard epifluorescence microscopy, the behavior of GFP-tagged microtubules (MTs) was examined in living hyphae of the *ropy-1* (dynein) and *ropy-3* (dynactin) mutants of *Neurospora crassa* and compared to a strain wild type for both mutations. Images were assembled in space and time for a precise picture of the 3-D organization of the microtubular cytoskeleton and a clear view of its dynamics. In wild-type *N. crassa*, cytoplasmic MTs were mainly arranged longitudinally along the hyphal tube. Straight segments were rare; most MTs showed a distinct helical curvature with a long pitch and a tendency to intertwine with one another to form a loosely braided network throughout the cytoplasm. In the apical and subapical zones, there was a high density of MTs. In both *ropy-1* and *ropy-3* mutants, there was a marked decrease in the number of MTs along the distorted hyphal tubes. This decrease was especially evident in the apical region. In the *ropy* mutants, MTs were generally shorter than in the wild type and showed a greater tendency to form thick bundles. Overall, the microtubular cytoskeleton of the *ropy* mutants appeared scant and disorganized. In the 3-D images, the helical character of MTs was evident but pitch and orientation relative to the growing axis fluctuated widely giving rise to the obliquely or transversely oriented segments of MTs seen frequently on 2-D images of the *ropy* mutants. As hyphae elongated, the MTs moved forward in a helical pattern that was more readily apparent in the mutants because of the fewer number of MTs. In conclusion, the dynein or dynactin deficiencies of the *ropy* mutants cause a severe perturbation in MTs organization, as previously described, but also in microtubule polymerization and dynamics with serious negative consequences on organelle motion and distribution and hyphal growth rate.

Localisation of the putative polarisomal component Spa2 in *Aspergillus niger*

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The ability of cells to polarize is essential during morphogenesis in yeasts and filamentous fungi. Cytoskeletal components, including actin, are thought to play an important role in polarized growth. It has been shown in yeast that the actin-organizing polarisomal component Spa2 localizes to sites of polarized growth, demonstrating that Spa2 can be used as a marker for polarization in yeast. Having such a marker for the filamentous fungus *Aspergillus niger*, would allow us to examine the localisation of polarisomal components in growing hyphae.

We searched the *A. niger* genome and identified a *spa2* homologue that encodes a protein of 908 amino acids. This protein is the only one in the genome that shares significant homology with the yeast homologues and we thus designated it Spa2. In order to examine its cellular localisation, Spa2 was tagged with eCFP at its C-terminus and integrated into the genome of the *A. niger* strain via a gene-replacement with the endogenous *spa2* gene. Thus, the fusion protein is expressed under the control of its own promoter.

Transformants, carrying one, two or three copies of Spa2::eCFP were indistinguishable from the recipient strain with respect to morphology and growth rates, suggesting that the fusion protein is functional. Using fluorescence microscopy, we could localize Spa2::eCFP in germ tubes and hyphae of *A. niger* at sites of polarized growth. The fusion protein typically appeared as a crescent, a localisation pattern conforming to that found in yeasts, suggesting that the *A. niger* Spa2 may play a similar role in polarised growth. However, the copy number of *spa2::ecfp* was critically important for detecting the fusion protein. Whereas a single copy of *spa2::ecfp* resulted in poor brightness, strains carrying two copies expressed Spa2::eCFP at a level sufficient for imaging. In strains harboring at least three copies, Spa2::eCFP was increasingly detected within the cytoplasm, indicating that Spa2 becomes aberrantly localised when overexpressed.

IVp-9

Two approaches for the identification of *Aspergillus niger* genes involved in polar growth and branch initiation

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Analysis of protein secretion in *Aspergillus niger* revealed that proteins are mainly secreted from hyphae at actively growing tips, suggesting that protein secretion and hyphal tip growth are coupled processes. This has led to the suggestion that hyperbranching mutants displaying an increased apical surface have the potential to significantly secrete more protein of interest. To modify and optimize the morphology of *A. niger* by rational genetic engineering, it is important to know which regulatory networks control polarized growth and determine morphological characteristics of *A. niger*.

One approach in which those networks can be identified, is to study the genome-wide transcriptional changes in *A. niger* germlings following exposure to morphology-affecting compounds. We have therefore screened a series of compounds and followed their effect on germ tube elongation and branching by microscopy. In response to the antifungal drugs caspofungin, fenpropimorph and AFP, tip splitting as well as subapical branching were observed, suggesting that these compounds affect polarity establishment and maintenance in *A. niger*. Thus, measurement of changes in gene expression upon exposure to those drugs can help to discover key genes involved in regulation of polar growth.

Another strategy we embark on focusses on a temperature-sensitive hyperbranching mutant of *A. niger* (*ramosa-1*; Reynaga-Pena and Bartnicki-Garcia, 1997). We could show that germlings of *ramosa-1* grown at permissive temperature display a wild-type morphology, but branch apically and subapically when shifted to the restrictive temperature. Hence, with the comparison of the transcriptome of *ramosa-1* with the transcriptome of the parental strain it will also be possible to uncover the network of genes involved in polarity establishment and maintenance.

Reynaga-Pena CG and Bartnicki-Garcia S (1997) Apical branching in a temperature-sensitive mutant of *Aspergillus niger*. *Fung Genet Biol* 22: 153-167

Structure and function of the septal pore cap (SPC) in Basidiomycetes.

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Ascomycete and basidiomycetes hyphae consist of cytoplasmic compartments that are separated by perforated septa, generating a continuous system. Septa are crucial for important processes such as differentiation (e.g. sporulation) and damage control (pore-plugging) but they may have other functions as well. Ascomycetes are characterized by relatively simple structured septa, while those of basidiomycetes are generally more complex. Basidiomycete septa contain a characteristic rim around their central pore, the dolipore, which in higher basidiomycetes is often covered by a septal pore cap (SPC). Although SPCs were already observed in 1958, their composition and function is still unknown. To understand the function of the SPC of the model basidiomycete *Schizophyllum commune*, we purified these organelles using discontinuous sucrose gradients and filtration. The characteristic dome shape of the SPC was maintained during the whole process as was shown by Transmission Electron Microscopy. SDS-PAGE analysis of the purified SPCs revealed an abundant 14 kDa protein. The N-terminal sequence of this protein was determined and matched with a gene in our *S. commune* genomic database. Interestingly, homologues of this gene could only be found in fungi that also possess SPCs. We have raised antibodies against the 14 kDa protein and are currently using them for immuno-localization of the protein. We also intend to inactivate this gene.

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Identification of an acidic protein that accumulates during sexual development in *Sordaria macrospora* and *Neurospora crassa*

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It was shown several years ago that an acidic protein accumulates in perithecia from several *Neurospora* species, but the identity of the protein and the encoding gene remained unknown. We have now isolated a protein from *Sordaria macrospora* showing similar biochemical features: The protein appears as a fast-migrating band on native gels of perithecial extracts but is absent from mycelial protein preparations. We have obtained peptide sequences from the *S. macrospora* protein by mass spectroscopy and were able to identify the corresponding gene which is termed *app1* (*abundant perithecial protein 1*). Interestingly, *app1* was identified previously by microarray hybridization as being strongly downregulated in three developmental mutants from *S. macrospora*. Additionally, not only the protein but also the transcript is observed only under growth conditions that permit sexual development. A *Neurospora crassa* knockout strain in the corresponding orthologue *app-1* does not accumulate APP-1 protein as expected; however, the knockout is fertile in homozygous crosses as well as in crosses with the wild type both as male and female partner. In heterozygous crosses of the *app-1* knockout with the wild type, APP-1 protein accumulates only when the wild type is the female parent, not when the knockout strain is used as female, indicating that the protein is formed by the vegetative tissue of the fruiting body.

Genetic control of hyphal morphology in *Aspergillus nidulans*

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The molecular biology behind the development of *A. nidulans* hyphae has been studied for many years. Although the physiology of mycelia can be altered by varying the growth medium, it has been shown that there is very strong genetic control of the process of mycelial development.

The HbrB protein was identified in a screen for temperature sensitive mutants. It has no known domains and orthologues are detectable only in some closely related fungal species. When grown at a restrictive temperature cells do not show much apical extension, but produce many lateral branches and exhibit hyperseptation. An *alcA-hbrB* strain was created previously and under promoter repressing conditions growth is stunted and the cells are very swollen. A strain containing HbrB fused to GFP has been created and localisation of the protein studied.

The *Aspergillus nidulans* homologue of *N. crassa cot-1*, *cotA*, encoding a member of the NDR protein kinase family, has previously been cloned and expressed under the control of the *alcA* promoter. Depletion of CotA by repression of the *alcA* promoter led to a severe growth defect accompanied by loss of polarity. A functional CotA-GFP fusion protein has been created and was found in punctate regions of fluorescence similar to the distribution reported for the human homologue NDR2.

Co-localisation studies of CotA and HbrB with other tagged proteins may aid the identification of the subcellular compartments in which they reside. The *A. nidulans* homologue of Vps52 (AN4014.2), a member of the Golgi Associated Retrograde Protein (GARP) complex has been tagged with both GFP and RFP. This protein has been shown to localise to the Golgi in both *Saccharomyces* and *Arabidopsis* and can now be used as a marker for Golgi in *A. nidulans*.

These new strains will facilitate further characterisation of the two proteins and indicate their role in the control of hyphal development in *A. nidulans*.

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The role of BemA in the genetic control of *Aspergillus nidulans* hyphal morphology

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The publication of the *A. nidulans* genome sequence has given us the opportunity to identify homologues of genes from other species that have been shown to be involved in the process of polar growth. Although there will be gaps in the data, which is inevitable because of the differences in how the organisms grow, it is expected that many of the fundamental mechanisms will be similar.

BemA, the *A. nidulans* orthologue of the *S. cerevisiae* protein Bem1p was identified through genome sequence comparison and we have shown that it plays a similar role to that in yeast, where it acts as a scaffold protein involved in cell polarity establishment. BemA appears to be essential for the establishment of cell polarity and has a major role in the maintenance of polarity once an axis has been established. We have shown that the first SH3 domain of the protein is not essential for normal cell growth and have evidence to suggest alternate transcriptional start sites.

The BemA protein was 3' tagged with RFP in order to visualise its location in the cell. BemA is first observed around the nucleus and it is also seen around the cell periphery. In early germlings the protein begins to accumulate at the hyphal tip, eventually forming a well-defined cap. These caps can also be seen at the tips of branches. Its presence around the nucleus in germinating conidia may reflect localisation in the endoplasmic reticulum prior to its re-location to the points of growth.

Sexual crosses have been performed to introduce BemA-RFP into several mutant backgrounds to observe the location of BemA when these strains are exhibiting defects in polarity. This may give us an insight into the role of these proteins in hyphal development and the interactions between them.

Formation of aerial hyphae, attachment and pathogenicity in *Ustilago maydis* in the absence of hydrophobins

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Ustilago maydis contains one repellent and two hydrophobin genes in its genome. The repellent gene *rep1* has been described previously. It encodes 11 secreted repellent peptides that result from the cleavage of a precursor protein at KEX2 recognition sites. The hydrophobin gene *hum2* encodes a typical class I hydrophobin of 117 aa, while *hum3* encodes a hydrophobin that is preceded by 17 repeat sequences. These repeats are separated, like the repellent peptides, by KEX2 recognition sites. Gene *rep1* was lowly expressed in a wild-type haploid grown on minimal medium but was highly expressed on a plant surface. High expression of *rep1* was also observed when haploid strains lacking the *uac1* gene or expressing an active b-complex were grown on minimal medium. Hydrophobin gene expression was very low, if present at all. Deletion of *rep1* affected formation of aerial hyphae, surface hydrophobicity as well as attachment to hydrophobic surfaces. In contrast, deleting either or both hydrophobin genes only affected aerial hyphae formation. From these results it is concluded that hydrophobins of *U. maydis* have been functionally replaced, at least partially, by repellents and possibly other molecules as well.

IVp-15

Strobilurins and sex in *Mycosphaerella graminicola*

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Over the past three seasons, resistance to strobilurin fungicides has rapidly evolved in the foliar wheat pathogen *Mycosphaerella graminicola*. This resistance is conveyed by a point mutation in the cytochrome b gene in the mitochondrial genome. In order to study the inheritance of strobilurin resistance, we crossed resistant and sensitive isolates of *M. graminicola* on wheat seedlings that were pre-treated with azoxystrobin (Amistar). Three such crosses were made at each of the following concentrations based on the full recommended dose: 0% (control), 3%, 6%, 12.5%, 25%, 50%, 100%, and 200%. Ratios of resistant to sensitive progeny were determined by comparing the number of germinating vs. non-germinating ascospores after discharge onto water agar versus water agar amended with 1 ppm azoxystrobin in a total of over 17,000 ascospores counted. These ratios were confirmed by a diagnostic strobilurin resistance PCR screen on more than 1,000 isolates from a random collection of over 3,000 ascospores discharged onto unamended water agar. To our surprise, all crosses were successful under all preventive applications of azoxystrobin. Hence, in planta, sensitive isolates can very effectively and consistently overcome the disruption of mitochondrial respiration and even participate in sexual reproduction under the aforementioned fungicidal pressure range. We observed a strong maternal/paternal preference in control crosses on untreated plants as indicated by segregation ratios in progeny of approximately 1:0 or 0:1 for strobilurin resistance : sensitivity. When there was no maternal preference for the strobilurin resistant isolate, a pre-treatment of just 6% of the full recommended dose of azoxystrobin was sufficient to shift the progeny to nearly all resistant. We hypothesize that this is an example of stress-induced male behavior. These findings can help explain the unexpected rapid development and distribution of strobilurin resistance in the Western European *M. graminicola* population.

Vegetative incompatibility in *Aspergillus niger*

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In filamentous fungi, anastomosis between hyphal filaments is essential to fungal growth and occurs both within and between individual colonies. As a consequence of the latter, heterokaryotic cells may arise, the viability of which is determined by heterokaryon incompatibility (*het*-) genes: allelic differences for either of the *het*-loci results in cell death. For some of the ascomycetes (e.g. *Aspergillus nidulans*, *Neurospora crassa* and *Podospora anserina*) genetic analysis revealed the number of unlinked *het*-loci involved in the vegetative self/non-self recognition to vary between species from 5 to 11. In addition, there are downstream effectors of death during heterokaryon incompatibility. E.g. in *P. anserina* a set of genes termed *idi* (induced during incompatibility) were found that were also involved in autophagy.

For *A. niger* heterokaryon incompatibility was observed among natural isolates as the inability to complement recessive nuclear mutations in a heterokaryon and to transfer a mitochondrial marker. In addition, heterokaryon incompatibility prevented the spread of dsRNA viruses that were found in approximately 10% of the natural isolates of black Aspergilli. However, genetic analysis in this asexual fungus is impossible by definition because parasexual analysis requires heterokaryon formation. As a result it is formally not known whether heterokaryon incompatibility in this asexual fungus is under a similar genetic control to that found in related sexual species. Moreover, in the absence of recombination the maintenance of heterokaryon incompatibility depends on mutation to compensate for loss of incompatibility types by random drift.

In this study we investigated the *A. niger* database for the main proteins involved in the heterokaryon incompatibility reaction in either of the following sexual species: *A. nidulans*, *A. fumigatus* (recently found to be sexual), *N. crassa* or *P. anserina*.

IVp-17

Genetic regulation of conidiation in *Trichoderma* has major parallels to the *Neurospora* model

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Trichoderma species are ubiquitous soilborne Ascomycetes noted for their biocontrol capabilities against plant pathogens as well as industrial cellulase production. A characteristic feature of *Trichoderma* species is the production of concentric conidiation rings in response to alternating light-dark cycles. This response has been shown to be due to blue and/or UVA light, which is regulated by the Blue Light Regulators Blr1 and Blr2 (orthologues of the *Neurospora crassa* White Collar proteins) (Casas-Flores *et al.*, 2004). In this study we have isolated orthologues of *N. crassa rco1* (Regulator of Conidiation) and *frq* (Frequency) from *T. atroviride* and *T. hamatum* and are investigating their role in conidiation. In *N. crassa* the conidiation gene *con-10* is expressed in mycelia of *rco1* deletion mutants, suggesting negative regulation of conidiation genes by Rco1, during vegetative growth. Experiments are currently underway to create *rco1* knockdown mutants in *T. atroviride* and to isolate a *con-10* orthologue. Northern analysis of *con-10* in the resulting transformants will be used to investigate whether *rco1* has a similar role in *Trichoderma* to the role it is proposed to have in *Neurospora*.

As part of this study a series of experiments investigating the effect of light, injury and ambient pH on conidiation was performed revealing evidence for a circadian rhythm. In *N. crassa* the White Collar proteins form a circadian clock in conjunction with the protein Frequency. We have recently isolated an orthologue of the *N. crassa frq* gene from *T. atroviride* and *T. hamatum*. Experiments are currently underway to investigate whether the expression patterns of *frq* in *T. atroviride blr* mutants is similar to that of *frq* in *N. crassa* white collar mutants, which would suggest the presence of a WC/FRQ circadian clock in *Trichoderma*. These and other experiments will be discussed.

Casas-Flores S., Rios-Momberg, M., Bibbins, M., Ponce-Noyola, P. & Herrera-Estrella, A. (2004). BLR-1 and BLR-2, key regulatory elements of Photoconidiation and mycelial growth in *Trichoderma atroviride*. *Microbiology* **150**: pp. 3561-3569.

Comparative gene expression analysis of fruiting body development in two distantly related filamentous fungi

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The ascomycete *Pyronema confluens* (Pezizales) readily forms fruiting bodies under laboratory conditions. Here, we report the first molecular analysis of fruiting body development in this filamentous fungus. Phylogenetic analysis with the combined sequences of 15 genes from *P. confluens* and 11 other fungi placed *P. confluens* at the base of the filamentous ascomycetes. Two *P. confluens* cDNA libraries were generated, one derived from mycelium undergoing sexual development, the other from vegetative mycelium. From each library, 96 clones were end-sequenced, resulting in the identification of 132 different genes. Expression studies of ten *P. confluens* genes by quantitative real time PCR identified seven genes that are transcriptionally up- or downregulated during sexual development when compared to vegetative growth. As a first step towards a comparison of gene expression during fruiting body development in different filamentous fungi, transcript levels of the corresponding homologs from *Sordaria macrospora* were analyzed by quantitative real time PCR. *S. macrospora* is a member of the Sordariales, a derived group of ascomycetes, and has frequently been used as a model system for fruiting body development. The analyses revealed conserved expression patterns during sexual development for several of the *S. macrospora* genes when compared to *P. confluens*. One of the genes that is upregulated during fruiting body formation in *S. macrospora* and *P. confluens* encodes a COP9 signalosome subunit; and the corresponding *Neurospora crassa* knockout mutant is female sterile.

IVp-19

Trisporoids in sexual reactions of zygomycetes: inter- and intracellular signalling

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Trisporic acid and its precursors are active in partner recognition and in modulating the intracellular signal response in zygomycete mating reactions. Recognition occurs between compatible individuals within a broad systematical range, with several naturally occurring trisporoid derivatives being recognized by putative partners. Mating type-specific precursors modulate a number of response reactions, among them they induce the formation of sexually differentiated hyphae or hyphal units in the partner. Such reactions are best analysed by comparing the reactions of the highly sensitive *Mucor mucedo* towards diverse precursors and analogs (see presentation Mareike Richter).

Species specificity of the mating reaction is possibly accomplished only at the subsequent biosynthesis steps leading from the mating partner's precursor to trisporic acid, probably involving differentiation between the numerous derivatives and isomers by the metabolic enzymes. Two of these enzymes are hitherto known, both catalyzing oxidation reactions at C₄ of the ring moiety of the trisporoid molecule: 4-dihydromethyltrisporate dehydrogenase (TDH) produces methyl trisporate from the (+)-mating type-specific pheromone 4-dihydromethyl trisporate, and 4-dihydrotrisporin dehydrogenase (TNDH) converts 4-dihydrotrisporin into the (-)-mating type-specific pheromone trisporin. To facilitate analysis of TDH, a protocol for the maltose-tagged heterologous expression and subsequent activation of the enzyme has been optimized. The fusion product exhibits similar activities with both 4-dihydromethyl trisporate and 4-dihydrotrisporin, whereas natural TDH shows a preference for 4-dihydromethyl trisporate. The activity of *Mucor mucedo* TDH has also been tested with several other compounds and analogs. The ability to use both 4-dihydro-compounds as substrates has been verified for TDH from *Mucor mucedo*, *Phycomyces blakesleeanus* and *Absidia glauca*. In the first two species, a distinct second enzyme with TNDH activity exists, whereas TDH seems to be the only dehydrogenase in *Absidia glauca* trisporoid biosynthesis.

TDH activity is generally strong in young cultures or hyphal units, and becomes reduced with age and/or developmental status, but the exact expression of the enzyme activity differs between the different species. This indicates a time-controlled developmental scheme probably also involved in species specificity of mating reactions.

Using sequence information of *Rhizopus oryzae*, beta-carotene dioxygenase genes, probably involved in carotene degradation as initial step of trisporoid synthesis, were cloned from *Blakeslea trispora* and *Mucor mucedo*. Heterologous expression will allow for detailed analysis of the reaction and reaction products.

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- Schimek *et al.* (2005) *Fungal Genet Biol* 42, 804-812
- Schultze *et al.* (2005) *Gene* 348, 33-44

Perturbation of cell wall caused by defect of the KexB in *Aspergillus oryzae*.

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We previously isolated the *kexB* gene, which encodes a subtilisin-like processing enzyme, from a filamentous fungus, *Aspergillus oryzae*, and constructed a *kexB* disruptant (*delta-kexB*) to examine the physiological role of *kexB* in *A. oryzae*. The disruption of the *kexB* gene led to remarkable morphological defects on Czapek-Dox (CD) agar plates. We found that upregulation of transcription levels of *mpkA* and cell wall biogenesis genes in the *delta-kexB* strain is autoregulated by phosphorylated MpkA as the active form through cell integrity signaling. Because the KexB defect causes the constitutive activation of cell integrity signaling, biochemical analyses of cell wall structures of the disruptant contribute to understanding of the response reaction of the cell integrity pathway at the cell wall level and/or enable us predict cellular KeB-targets involved in cell wall biogenesis. In the present study, to reveal how the constitutive activation of the cell integrity signaling caused by the *delta-kexB* mutation affects structures and biogenesis of cell wall structures, we analyzed the organization and components of cell wall of *A. oryzae delta-kexB*. Contents of glucose and hexosamine in the cell wall of the *delta-kexB* strain grown on CD agar plates decreased to about 50% and increased to about 1.5-fold of those in the cell wall of the wild-type strain, respectively. The result suggested that the KexB defect caused alterations of contents of critical and major cell wall polymers such as beta-1,3-glucan (and/or alpha-1,3-glucan) and chitin. To analyze the structure of cell wall in the *delta-kexB* strain grown on the CD agar plates, cell wall was fractionated by alkaline extraction. The *delta-kexB* cells showed a significant decrease of the amount of alkali-soluble fraction compared with that of wild-type cells, whereas the amount of alkali-insoluble fraction prepared from the *delta-kexB* cells was slightly larger than that from wild-type. The alkali-insoluble fraction prepared from *delta-kexB* cells contained a larger amount of hexosamine than that from wild-type cells. Chitin synthase activity of *delta-kexB* cells was also higher than that of the wild-type cells. From these results, we speculated that the decrease of the alkali-soluble fraction in the *delta-kexB* strain was compensated with the increased chitin content by the high activity of chitin synthase through the activated cell integrity signaling.

IVp-21

Oxygenases from *Aspergillus niger* and their influence on sporulation and development

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A set of oxylipins called psi factors have been shown to influence the ratio of asexual to sexual sporulation in *Aspergillus nidulans*. Three genes encoding fatty acid oxygenases (*ppoA*, *ppoB* and *ppoC*) from this fungus have been related to this process¹.

We have demonstrated the occurrence of psi-producing oxygenases in the asexual ascomycete *Aspergillus niger* and have identified the oxylipins that possibly act as regulators of sporulation. Homologues of *ppoA*, *ppoB*, and *ppoC* have been identified for *A. niger*. Disruption strains were constructed for these genes and analyzed with respect to growth, sporulation and oxylipin content in comparison to a wild-type. Furthermore we studied the expression of genes that are known to be involved in the asexual and sexual cycle in *Aspergillus* in these strains to determine whether the link between the sexual and asexual cycle as determined in *A. nidulans* is also present in *A. niger*.

¹ Tsitsigiannis, D.I., Kowieski, T.M., Zarnowski, R., and Keller, N.P. (2005) Three putative oxylipin biosynthetic genes integrate sexual and asexual development in *Aspergillus nidulans*, *Microbiology* 151 1809-1821.

Two novel calcium channel mutants in *Gibberella zeae* affect ascospore development and discharge.

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Gibberella zeae (anamorph *Fusarium graminearum*) infects wheat, corn and numerous other crop plants to inflict substantial economic losses worldwide. The primary inoculum is the ascospore, forcibly discharged from perithecia which have developed on crop debris. Consequently, ascospore discharge is of considerable interest in understanding the disease cycle of this fungus. We have been investigating the mechanism and regulation of ascospore discharge by performing targeted gene knockouts. Recently, we have generated two mutants which affect ascospore development and discharge. The *mid1* stretch-activated calcium channel mutant produces apparently normal perithecia and asci, but lacks ascospores. Meiosis, followed by at least two mitotic nuclear divisions, takes place within the ascus, but spores do not fully develop. In the *cch1* voltage gated calcium channel mutant, phenotypically normal ascospores are formed, but these spores are never discharged. Vegetative growth is abnormal in this mutant, with a zonate, fluffy, slow-growing colony. MID1 and CCH1 have been examined in detail in yeasts, where they are believed to form separate parts of a single protein. Consequently, the differing phenotypes in *G. zeae* for the two mutants are of interest. This is the first time either gene has been characterized in a filamentous fungus.

IVp-23

Expression of monokaryon and dikaryon-specific promoters of *Schizophyllum commune* in *Pycnoporus cinnabarinus*

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The *SC3* hydrophobin gene of *Schizophyllum commune* was expressed in *Pycnoporus cinnabarinus*. Temporal expression of *SC3* in *P. cinnabarinus* was identical to that in *S. commune*. The *SC3* promoter was active in a monokaryon but not in a dikaryon. Expression in the monokaryon peaked sharply at day 3 in liquid shaken cultures, while expression was high both in 3-day and 6-day old cultures on solid media. Temporal expression of the promoter of the hydrophobin gene *SC4* of *S. commune* was not identical in *P. cinnabarinus* compared to homologous expression.

Expression of the laccase gene *lcc3-1* under control of the *SC4* promoter was found exclusively in dikaryons of *S. commune*, while in *P. cinnabarinus* both monokaryons and dikaryons expressed the gene. From these results we conclude that some mechanisms involved in mono- and dikaryon specific gene expression are conserved in the homobasidiomycetes.

Functional study of *SMR1/Mat1-1-2*, a mating-type gene which does not control self-non self recognition in *Podospora anserina*

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The sexual reproduction of *P. anserina* requires the coordinate action of 4 mating-type genes. Three of these encode transcriptional factors which control the recognition between *mat-/Mat1-1* and *mat+/Mat1-2* sexually compatible cells during fertilization, and between nuclei after fertilization, at the transition from a plurinucleate to a dikaryotic stage when ascogenous hyphae are formed. The fourth gene, *SMR1/Mat1-1-2*, is essential for development of the dikaryotic hyphae and its inactivation results in barren perithecia. By contrast, it is dispensable for development of abnormal uniparental ascogenous hyphae observed when the other *mat* genes contain mutations affecting nuclear pairing. *Mat1-1-2* is present in all Sordariomycetes analyzed to date. All MAT1-1-2 proteins contain a stretch of 17 aa with conserved and invariant residues (histidine, proline, glycine). This so-called HPG domain was proposed to define a new DNA-binding domain (1). However, the computing prediction of SMR1 localization and its subcellular localization by GFP tagging suggest that it has a cytosolic localization. This feature does not support the assumption that SMR1 may be a transcription factor and its molecular function is not elucidated yet. We have determined the function of 35 SMR1 alleles constructed by site-directed mutagenesis. We have found that deletion of the HPG domain leads to a mutant phenotype confirming its role in SMR1 activity. However, 11 aa of this domain including H, P and G have been changed to alanine without affecting SMR1 activity. Only replacement of tryptophane 193 to alanine confers a sterile phenotype. Suppressors restoring fertility of the *smr1W193A* mutant have been isolated. All of them affect *mat* genes and nuclear pairing, similarly to the suppressors previously isolated from a *SMR1* KO mutant (2). We expected to characterize new kinds of suppressors since the mutant protein was thought to be present in the missense mutant while it was absent in the KO mutant. In fact, the *SMR1* transcript was not detected in barren perithecia from a cross of the *mat+* strain with *smr1W193A*, which thus is like a null mutant. By replacing the 5' UTR of *SMR1*, we have constructed a strain expressing a mutant SMR1 protein, which is now suitable for selecting suppressors able to reveal new genetic interactions that could help to understand the role of *SMR1*.

1. Debuchy et al (1993) Mol Gen Genet 241:667

2. Arnaise et al (2001) Genetics 159: 545

IVp-25

The b-dependently regulated transcription factor Hdp1 is involved in cell cycle regulation and filamentous growth in *Ustilago maydis*

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Ustilago maydis is a phytopathogenic basidiomycete on corn. Pathogenic development in *U. maydis* is initiated by the fusion of two haploid, yeast like sporidia, that is mediated by the *a* mating type locus via a pheromone/receptor based system. In the resulting dikaryon, the *b* mating type locus controls the morphological switch to filamentous growth as well as the subsequent steps in pathogenic and sexual development. *b* encodes a pair of homeodomain proteins, termed bE and bW, which can form a heterodimeric complex that functions as a transcriptional regulator. One of the genes regulated by the bE/bW heterodimer is *hdp1* (homeodomain protein 1). It encodes a potential homeodomain transcriptional regulator and shows high expression already 2 hours after formation of the bE/bW complex. Deletion of *hdp1* does not alter pathogenicity or mating; however, $\Delta hdp1$ mutant strains are reduced in filamentous growth. In addition to b-regulation, *hdp1* expression is dependent on Rbf1, a b-dependently expressed transcription factor that is required for filamentous growth as well as pathogenic development. Interestingly, induced expression of *hdp1* can restore the filamentation defects in $\Delta rbf1$ strains; similarly, filamentous growth is induced in wild type cells. FACS analysis and nuclear distribution imply that the filamentation is accompanied by a cell cycle arrest in the G2 phase. Microarray analysis revealed that induction of *hdp1* leads to an altered expression of two genes connected to cell cycle regulation, *clb1*(b type-cyclin) and *put-pcl2*(cyclin containing proteins). *Clb1* is an essential gene that is required to enter S and M phase. It is anticipated that the downregulation of *clb1* accounts for the observed cell cycle arrest. Unlike *clb1*, *put-pcl2* is not essential, and no obvious phenotype was observed in haploid *put-pcl2* deletionstrains. When *hdp1* expression is induced in a $\Delta put-pcl2$ background, filamentation is drastically reduced when compared to a wildtype strain. However, the *hdp1* induced cell cycle arrest appears not be released by the *put-pcl2* deletion. Although $\Delta put-pcl2$ deletion strains display reduced filamentation on the plant surface, pathogenicity is not altered. Our data indicate that *put-pcl2* displays its function mainly in the filamentation induced by *hdp1*.

Cand1 of *Aspergillus nidulans*

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Targeted protein degradation by the 26S proteasome controls various cellular processes in eukaryotes. It requires protein ubiquitination by ubiquitin ligases (E3), like the SCF (Skp1-Cullin1-Fbox). Activity of the SCF is regulated by the Cand1 (cullin-associated and neddylation-dissociated), a regulator of assembly of cullin containing E3s that is lacking in bakers yeasts. We identified an *A. nidulans* gene called *cand1*, with deduced aa identity of 20% to the human protein Cand1. To investigate the functions of Cand1 we constructed a *cand1* deletion strain. This strain shows a red color phenotype, produces less conidia than the wild type and fruitbody formation is blocked at early nest stage. Cand1 interacts with the fungal counterparts of human Cul1 and Cul4 in the yeast two hybrid system, indicating a conserved molecular function to the human protein. Our results suggest that the system regulating targeted protein degradation at ubiquitination level is highly conserved from *A. nidulans* to humans. Therefore, *A. nidulans* can be used as a model organism to gain new insight into the biological function of the regulatory protein. First results on differential expression of Cand1 and localization at defined points of development will be presented.

IVp-27

LRG1 is required for hyphal elongation in *Neurospora crassa*

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In a screen for mutants defective in hyphal morphogenesis, temperature-sensitive mutants that result in the cessation of hyphal elongation and induction of hyperbranching at restrictive temperature were identified. One identified gene, *lrg1* (LIM-RhoGAP), encodes a protein containing 3 LIM domains, which are most similar to vertebrate paxillin LIM domains. Paxillin is a focal adhesion organiser, suggesting a putative role of these LIM domains in actin organisation. The GTPase activating domain of LRG1 suggests an involvement of Rho proteins in hyphal elongation. Deletion of *lrg-1* in heterokaryotic background showed an identical phenotype to temperature-sensitive mutant. Complementation studies showed that full length LRG1 is required for proper tip elongation. Domain analysis of LRG1 was done to identify the regions essential for LRG1 function. Point mutations in the 1st (C121S, C124S, C98L, C101S), the 2nd (H185V, C188S, C162S, C165A) and 3rd (C492S, C495S, C469G, C472S) LIM domain and combinations of these mutants indicate that multiple LIM domains are necessary for wild type growth. The GAP domain of LRG1 is essential for polar growth, concluded from loss of function by mutation K910A. LRG1 protein accumulates in distinct spots at the cortex of hyphal tips. To determine which Rho protein is regulated by the GAP domain of LRG1 *in vitro* GAP assays are intended. So far, all six RHO proteins identified in the genome of *N. crassa* were heterologously purified from *E. coli*. Taken together, these results suggest that both the LIM domains and the GAP domain of LRG1 are essential for proper tip elongation.

A constitutively activated Ras-GTPase alters mycelial growth in *Coprinopsis cinerea* and affects *B* mating type regulated phenotypes in dikaryons and fruiting body development

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Ras belongs to the family of small GTPases and acts as a molecular switch, turning off downstream signalling pathways in relation to GTP or GDP binding. A constitutively activated *ras* mutant in the basidiomycete *Coprinopsis cinerea* has been transformed into different monokaryons of *C. cinerea*. Aerial mycelium is reduced in these transformants but not production of aerial asexual spores (oidia). Mycelium growth is invasive and hyphal branching disturbed. In matings, we observed transfer of *ras*^{val19} transformed nuclei into compatible monokaryons. However, the dikaryons formed had unfused clamp cells that often start to re-grow into curly hyphae. Mycelial growth at the beginning is sparse and invasive and there is a strong selection on mutations reversing the mycelial growth to wild type. Fruiting bodies on certain dikaryons in sectors of *ras*-disturbed growth phenotype are of reduced size and spore formation is partially hampered. Primordia have an altered shape.

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IVp-29

***Aspergillus nidulans* class V and VI chitin synthases CsmA and CsmB, each with a myosin motor-like domain, mutually perform compensatory functions that are essential for hyphal tip growth**

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One of the essential features of fungal morphogenesis is the polarized synthesis of cell wall components. Chitin, a beta-1,4-linked homopolymer of *N*-acetylglucosamine (GlcNAc), is one of the major structural components of the fungal cell wall. Chitin synthases, membrane-bound proteins that catalyze the polymerization of GlcNAc from UDP-GlcNAc as a substrate, have been classified into at least six groups, classes I to VI, on the basis of the structures of their conserved region. Actin is concentrated at the growing apices and sites of septum formation, where cell wall synthesis or septal synthesis is active. The actin cytoskeleton provides the structural basis for cell polarity in *Aspergillus nidulans*, as well as in most other eukaryotes. *A. nidulans* class V and VI chitin synthases CsmA and CsmB, each with a myosin motor-like domain (MMD), are conserved among most ascomycete filamentous fungi. No orthologue exists in the genome of the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. The *csmA* null mutant showed remarkable abnormalities with respect to cell wall integrity and the establishment of polarity. We demonstrated that CsmA tagged with 9x HA epitopes localized near actin structures at the hyphal tips and septation sites, and that its MMD was able to bind to actin. Characterization of mutants bearing a point mutation or deletion in the MMD suggests that the interaction between the MMD and actin is not only necessary for the proper localization of CsmA, but also for CsmA function. In this study, we investigated the functional relevance of *csmA* and *csmB*. The *csmB* null mutants exhibited defective phenotypes that were partly similar to the *csmA* null mutant. Moreover, *csmA csmB* double-null mutants were not viable. Mutants in which *csmB* was deleted and the expression of *csmA* was under the control of the *alcA* promoter were viable but severely impaired in terms of hyphal growth under *alcA*-repressing conditions. We revealed that CsmB tagged with 3x FLAG epitopes localized at the hyphal tips and forming septa, and that the MMD of CsmB was able to bind to actin *in vitro*. These results suggest that CsmB is involved in hyphal tip growth and septum formation *via* the interaction between its MMD and the actin, according to a mechanism similar to that of CsmA, and that CsmA and CsmB mutually perform compensatory functions that are essential for hyphal tip growth.

Senescence and rejuvenation in *Podospora anserina*

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Biological ageing is controlled by a complex network of molecular pathways and interactions. Mitochondria, the organelles of energy transduction, play a major role in this network, which may be related to the production of reactive oxygen species (ROS) as Harman's free radical theory of ageing suggests or due to other mechanisms. We are still far from understanding the details about the precise mechanisms how mitochondria and interactions of these organelles with other cellular compartments and the environment are involved in the control of life span and ageing.

Podospora anserina is a filamentous fungus with a long history in experimental ageing research and a well demonstrated mitochondrial etiology of ageing. Coprophilous *P. anserina* is characterized by a genetically controlled short life span (approx. 25 days) and a short generation time (approx. 14 days). Classical genetic experiments revealed a clear genetic basis of ageing and specifically the involvement of extrachromosomal genetic factors in *P. anserina*. The demonstration that mitochondrial inhibitors affect life span clearly pointed to mitochondria as the cellular compartment involved in the control of the ageing process. Subsequently, molecular analyses revealed age-dependent reorganizations of the mitochondrial DNA (mtDNA) playing a central role in ageing of *P. anserina* wild-type cultures.

Ascospores produced on a (pre-) senescent culture will often display a juvenile phenotype (a phenomenon termed rejuvenation). To study whether and how this rejuvenation occurs, a comparison was made between the fitness of spores and the fitness of the mycelium. A full grown mycelium was sampled at five different time points during its lifespan and both samples of the mycelium and of produced ascospores were tested in turn for their lifespan. The results suggest intra-organismal selective access of functional juvenile mitochondria to reproductive structures.

IVp-31

Deneddylation activity of the COP9 signalosome is essential for *Aspergillus nidulans* sexual development

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The COP9 signalosome (CSN) is a conserved multiprotein complex, playing an essential role in development of eukaryotes through regulation of protein ubiquitination and degradation. All CSN subunits have been identified in the filamentous fungus *Aspergillus nidulans*. Considering the conservation of the proteasome-related machinery in this organism, it provides an easily amenable model for studying the broad array of CSN regulatory functions.

Deletion of the *csnE* subunit results in pleiotropic phenotypes affecting formation of fruit bodies during sexual development, sensitivity to oxidative stress and secondary metabolism. CSNE promotes cleavage of ubiquitin-like protein Nedd8 from the CULA subunit of the SCF ubiquitin ligase. We have examined the role of deneddylation by point mutagenesis of the JAMM metalloprotease motif in *csnE*. Analysis of mutant phenotypes indicates that intact JAMM motif is necessary to complete sexual development and its loss leads to the full mutant phenotype. Furthermore, as shown by Western blot, a modified (presumably neddylated) form of CULA is enriched in all *csn* mutant strains in early stages of *A. nidulans* development, indicating that CSNE acts as deneddylase in the fungus and that this function is required around the time of developmental competence. The role of the Nedd8 protein homologue and neddylation in *A. nidulans* are currently under investigation.

***Het-S* spore killing in *Podospora anserina*: Non-Mendelian nuclear inheritance caused by the non-Mendelian cytoplasmic HET-s prion-element**

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Two alleles of the *het-s/S* locus occur naturally in the filamentous fungus *Podospora anserina*, *het-s* and *het-S*. The *het-s* encoded protein can form a prion that propagates a self-perpetuating amyloid aggregate, resulting in two phenotypes for the *het-s* strains. The prion infected [Het-s] shows an antagonistic interaction to *het-S* whereas the prion-free [Het-s*] is neutral in interaction to *het-S*. The antagonism between [Het-s] and *het-S* is seen as heterokaryon incompatibility at the somatic level and as *het-S* spore killing in the sexual cycle. The consequences of the unique transition from a coenocytic to a cellular state in the sexual phase and the timing and localization of paternal and maternal HET-s and HET-S expression that are pertinent to prion transmission and *het-S* spore killing are elaborated. A model for *het-S* spore killing is proposed.

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Morphogenesis of filamentous fungi due to the impact of fluid dynamics

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A broad range of products is obtained by industrial cultivation of filamentous fungi. Difficulties occur with their complex morphology - growing pelleted or as free dispersed mycelia. The production rate of a desired product can vary significantly with fungal morphology. Process optimisation should reflect the mechanisms involved in fungal morphogenesis, which in turn needs to be represented by adequate models. Investigation of the growth of *A. niger* showed that conidial aggregation strongly depends on the amount and consistence of particles at the onset of cultivation. A first aggregation of spores takes place immediately after inoculation leading to a steady state of formation and disruption of conidial packages. The influence of fluid dynamic conditions and pH-dependent surface forces on this process was studied. A second stage of aggregation is triggered by hyphal growth when emerging germ tubes provide new surface for conidial attachment. Hyphal length growth rate seems to play a decisive role in this second aggregation. About 20 h after inoculation the cultivation with *A. niger* conidia, pellet formation out of aggregates is completed. A complex structure of bimodal pellet size distribution is achieved in a consequence of elongation and branching of single hyphal elements and shaved off outer hairy hyphal elements which are able to form new, smaller pellets leading to altered pellet density. Each process, conidial aggregation, hyphal growth and pellet formation, can be described by dynamic models based on population balances. In the near future, the focus will be laid on analysing the particle size distributions in the time frame between the second stage of aggregation and the fully developed biopellets. To get the holistic pictures, methods like digital image analysis, focused beam reflectance measurements and laser diffraction are employed. These investigations are necessary to understand the processes which distinguish between pelleted or free dispersed mycelium. Furthermore, a mathematical description is essential for a sophisticated process development for filamentous growing fungi.

Mitotic crossing-over in chromosome III of the asexual fungus *Aspergillus niger*; how well does parasexual analysis correlate to the physical map?

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Aspergillus niger has many uses in food applications, for example as a producer of organic acids and enzymes. Strain improvement has thus far mainly been achieved by subsequent rounds of mutations and selection, and in some cases by the recombination of independent mutations located on different linkage groups. Another use of the parasexual cycle is the construction of diploids with improved characteristics. *A. niger* is an asexual fungus, but using the parasexual cycle, strains can be combined into heterozygous diploids, and upon haploidization, different combinations of unlinked mutations can easily be obtained. However, the exchange of mutations positioned on the same chromosomes, requiring mitotic crossing-over or gene conversion in the diploid phase, is very rare.

In order to study crossing-over, we improved the genetic map of chromosome III using the physical map. Using 6 auxotrophic markers, we constructed linkage group III specific marker strains. In a diploid containing five of these markers, we tested various effectors that could influence recombination of linked markers during haploidization and compared our findings with the physical map of chromosome III. In addition, we studied the mitotic crossing-over frequencies by selection of homozygous *cnxD* diploids from a heterozygous diploid on chlorate. Chromosomal locations with increased mitotic cross-overs were searched for patterns possibly involved in recombination.

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The course of fruiting body development in the basidiomycete *Coprinopsis cinerea*

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Coprinopsis cinerea (formerly *Coprinus cinerea*) is used as a model fungus for studying fruiting body development, mainly because of the ease to get fructifications in the laboratory within a short time. Another reason to study this fungus is the synchronized karyogamy and meiosis occurring within the basidia on the gills of the mushrooms. Stages of fruiting body development have been described in the past by Buller (1910), Matthews and Niederpruem (1972, 1973), Lu (1974), Reijnders (1979) and Moore and co-workers (1979, 1998). However, nowhere in the literature the full process of fruiting body development is documented. Particularly, the initial stages of primary and secondary hyphal knots and tissue development in the primordia are little studied. Fruiting body development in *C. cinerea* is a very complex process. Long observation training is needed to unequivocally identify and define the different developmental stages from primary hyphal knots to mushroom autolysis. Here, we present a picture catalogue of stages in fruiting body development of *C. cinerea* with the main cytological-physiological events in tissue formation and differentiation and basidia and spore development.

Laccase and peroxidase activities during growth and fruiting development of *Pleurotus ostreatus*

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Pleurotus species are the second most cultivated mushrooms in China (annual production of around 2.5 Mio tones) and probably also in the world. The fungi are cultivated on straw, sawdust and other lignocellulosic wastes. The edible white rot-fungus *Pleurotus ostreatus*, also known as oyster mushroom or hiratake, produces several ligninolytic enzymes for degradation of lignocellulosic substrates. The probably most important ones concerning lignin degradation are peroxidases and laccase. In this study, we used commercial wheat straw substrate blocks for the cultivation of two *P. ostreatus* strains and observed the activity of the extracellular enzymes MnP (manganese dependent peroxidase), MiP (manganese independent peroxidase) and laccase within the substrate during controlled cultivation over a period of 48 days. All three measured enzyme show the same pattern of developmental regulation. Enzyme activities within the straw follow rhythmic cycles with high production of enzymes in growth phases and drops in activity along with flushes of fruiting.

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Aberrant fruiting body formation in *Coprinopsis cinerea*: the etiolated stipe phenotype

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Homokaryon AmutBmut is a self-compatible strain that forms fruiting bodies without prior mating to another compatible monokaryon. Recessive gene defects in fruiting body development can be studied in this genetic background. A certain class of recessive mutations blocks normal tissue development within primordia. So called etiolated stipes (also known as dark stipes) with a poorly developed cap and a lengthy stipe form in the normal day/night rhythm. Such structures normally only develop when a culture is kept fully in the dark after a short light signal induced fruiting. Four different mutations are known to cause an etiolated stipe defect (*dst1*, *dst2*, *dst3* and *dst4*). Here, we compare the tissue structures of etiolated stipes formed on the mutants in light with those of etiolated stipes formed by homokaryon AmutBmut in the dark. Furthermore, we show that the two genes *dst1* and *dst2* affect light-induced asexual sporulation unlike genes *dst3* and *dst4*.

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Sporulation in *Stagonospora nodorum*

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Stagonospora nodorum is a necrotrophic fungal pathogen that is the causal agent of leaf and glume blotch on wheat. Some details of the early stages of infection have been revealed using gene disruption techniques, but very little is known in this, or any other pathogen, of the molecular basis of in planta sporulation. *S. nodorum* is a polycyclic pathogen. Rain-splashed pycnidiospores attach to and colonise wheat tissue and subsequently sporulate within 2-3 weeks. Several cycles of infection are needed to build up inoculum for the damaging infection of flag leaves and heads. Sporulation is therefore a critical component of its infection cycle; our aim is to determine the genetic and biochemical requirements for sporulation to allow more efficient control of the pathogen. Disease progression of *S. nodorum* on wheat cv. Amery was monitored by light microscopy to determine the time point when pycnidia development began. Early pycnidia development was evident 12 days post-infection. This information was used to guide a metabolomics based approach to determine requirements for sporulation in *S. nodorum*. Wheat plants were infected with *S. nodorum* and then polar metabolites extracted from the infected tissue. Rapid changes in the abundance of metabolites were detected during the onset of sporulation. Key fungal metabolites identified include mannitol and trehalose. The concentration of both mannitol and trehalose increased dramatically in concert with pycnidia formation. Both mannitol and trehalose have also been linked to pathogenicity in filamentous fungi. Creation of deletion mutants of the gene encoding trehalose 6-phosphate synthase, alone and in combination with a mannitol 1-phosphate dehydrogenase mutant are underway.

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A proteomic approach to characterise the function of the RNA-binding protein Rrm4 from the pathogen *Ustilago maydis*

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Active transport of mRNAs as ribonucleoprotein (RNP) particles along the cytoskeleton is a common mechanism for local restriction of protein synthesis and is essential for the establishment or maintenance of cell polarity during differentiation and development. In the smut fungus *Ustilago maydis* it has recently been shown that the RNA-binding protein Rrm4, which is part of shuttling RNP particles, is involved in determining cell polarity. The *rrm4* deletion strain is impaired in the formation of empty sections which are essential for polar growth of the infectious filament. To characterise the molecular defect caused by deletion of *rrm4* we chose a comparative proteomic approach with 2D PAGE. Already in pilot experiments the membrane-associated protein fraction revealed promising differences between filamentously growing wildtype and mutant strains. Using 2D DIGE technology we were able to specify ten significant differential expressed proteins. Using MALDI-TOF massspectrometry we identified protein species of the endochitinase Cts1 and the homolog of the Diamond Blackfan Anemia-associated Rps19 ribosomal protein. Our results demonstrate that the deletion of *rrm4* affects only a small subset of proteins which is indicative for a specific RNA transport. We suggest that Rrm4 is involved in membrane-associated translation which seems to be required for local cell wall remodelling as well as septa formation. Furthermore a novel extraribosomal function of Rps19 might be discussed.