Poster Category 1: Fungal Cell Biology

PR1.1

Role and spatio-temporal regulation of Rho1 in *Candida albicans*

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Small Rho G proteins such as Rho1 and Cdc42 are key regulators of the actin cytoskeleton. In *Saccharomyces cerevisiae*, Rho1 plays a critical role in cell wall integrity *via* beta-1,3-glucan synthase, protein kinase C (Pkc1) and the actin cytoskeleton. In *Candida albicans* Rho1, which has greater than 80% sequence identity with its *S. cerevisiae* and human counterparts, was shown to be essential. Using mutants, in which the sole copy of *RHO1* is under the control of the repressible Tetracycline promoter, we investigated the role of Rho1 in this organism. *PTEToffRHO1/rho1Δ* cells form hyphae in liquid media containing serum, but are defective in filamentous growth in embedded media and on solid media containing serum, indicating that Rho1 is required for invasive filamentous growth. As both Rho1 and Cdc42 are critical for filamentous growth, we set out to examine the spatio-temporal regulation of these proteins, under different growth conditions. Previous studies in *S. cerevisiae* have identified GTPase binding domains (GBD) that specifically bind activated G-protein: the Pkc1 Rho Interaction Domain (RID) has been used to localize activated Rho1, while Cdc42/Rac-Interactive Binding (CRIB) domain from Gic2 was used to localize activated Cdc42. We used similar GBDs, *i.e* a fusion of the RID from *C. albicans* Pkc1 with GFP to visualize activated Rho1, and the *S. cerevisiae* Gic2 CRIB domain fused to GFP, as Gic2 is absent from the *C. albicans* genome, to visualize activated Cdc42. The localization and dynamics of these sensors in wild-type cells and different mutants will be presented.

PR1.2

Systematic analysis of kinase and phosphatase function in *Candida albicans* yeast to hyphae transition

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An important factor in the pathogenicity of *Candida albicans* is its ability to exhibit a large morphological variability in response to changing environmental conditions. In particular, the morphogenetic switch between the yeast and hyphal form is thought to be an important virulence trait, helping the organism to gain access to and to proliferate in new host niches. To elucidate new genes that regulate this yeast to hyphae transition we systematically analyzed all identifiable protein kinases, phosphatases and their regulators in this morphogenetic process. We have used an inducible expression strain library to identify proteins that, when expressed, promote or inhibit the yeast to hyphal transition. The resulting library comprises a total of 224 strains, covering 123 verified and putative kinases, 39 phosphatases, 25 kinase and 6 phosphatase regulators. In addition to these wildtype genes, >30 mutant alleles were generated. After screening we have initially identified 22 different proteins that induce filamentation. Among these 22 were 12 proteins that have been previously shown to be involved in hyphal morphogenesis, including members of different MAP kinase cascades and cell cycle regulators. In addition to these previously characterized genes, our screen has identified 10 genes whose role in *C. albicans* filamentation has not been described. Currently, we are examining the molecular functions of these proteins in the yeast to hyphal transition and whether they function in previously described pathways or define novel pathways. The analyses of 2 of these protein kinases will presented.

PR1.3

Disruption of the *Trichoderma atroviride Eng18B* ENGase gene affects growth, conidiation and antagonistic ability

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The mycoparasitic fungus *Trichoderma atroviride* is known for its ability to antagonize plant-pathogenic fungi and is therefore used as a biocontrol agent in agriculture. The phylogenetic subgroup B5 of fungal glycoside hydrolase family 18 genes (GH18) encodes enzymes with mannosyl glycoprotein endo-*N*-acetyl-β-D-glucosaminidase (ENGase)-type activity. However, the biological roles of these enzymes are not known. The present work is a functional study of the *T. atroviride* Eng18B ENGase. Lack of N-terminal secretion signal peptide, transmembrane domains, or C-terminal GPI-anchor signal suggested a cytosolic localization of Eng18B. Gene expression analysis showed that *Eng18B* was induced in dual cultures with the fungal plant pathogens *Botrytis cinerea* and *Rhizoctonia solani*, although a basal expression was observed in all growth conditions tested. *Eng18B* disruption strains had reduced growth rates, but higher conidiation rates compared to the wild-type. However, growth rates on abiotic stress media were significantly higher in *Eng18B* disruption strains. No difference in spore germination, germ-tube morphology or in hyphal branching was detected. In addition, we determined that Eng18B is required for the antagonistic ability of *T. atroviride* against the grey mould fungus *B. cinerea* in dual cultures and that this reduction in antagonistic ability was partly connected to a secreted factor. These phenotypes were recovered by re-introduction of an intact *Eng18B* gene in *Eng18B* disruption strain. A putative role of Eng18B ENGase activity in the endoplasmatic reticulum associated protein degradation pathway (ERAD) of endogenous glycoproteins in *T. atroviride* is discussed in relation to the observed phenotypes.

PR1.4

Dual targeting of glycolytic enzymes to peroxisomes and cytoplasm is widespread in fungi

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In fungi, peroxisomes are not only important for metabolism of long-chain fatty acids but also for biosynthesis of cofactors and secondary metabolites. We observed that in the basidiomycetous fungus *Ustilago maydis* two core enzymes of glycolysis, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK) show dual localization in the cytoplasm and peroxisomes. Both enzymes contain hidden peroxisomal targeting signals (PTS1), which are unveiled by alternative splicing or ribosomal readthrough. *U. maydis* mutants lacking peroxisomal targeting of GAPDH were reduced in virulence. In addition, these mutants displayed a significant growth defect if other NADH dependent dehydrogenases were excluded from the peroxisomes. Dual targeting of glycolytic enzymes via cryptic peroxisomal targeting signals was also observed in other fungi suggesting an evolutionary conserved function of glycolytic enzymes in peroxisomes. Interestingly, a variety of different mechanisms operate in different species to achieve dual targeting.

PR1.5
Abstract Ref.617
Towards the extent and meaning of fungal alternative splicing
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During gene expression of higher eukaryotes, alternative splicing (AS) can produce various isoforms from one primary transcript. Thus, AS is thought to increase a cell's coding potential from a limited gene inventory. Although AS is common in higher plants and animals, its extent and use in fungi is mostly unknown. We undertook a genome-wide investigation of alternative splicing in 28 fungal species from the three phyla Ascomycota, Basidiomycota and Mucoromycotina, applying current bioinformatics data mining techniques. Our analysis reveals that on average over the investigated fungi, 6.2% of the genes are associated with AS. *Cryptococcus neoformans* and *Coccidioidis immitis* show outstanding rates of 18% and 13%, respectively. Intron retention is the predominant AS type in fungi, whereas exon skipping is very rare. The investigated Basidiomycota have on average higher AS rates (8.6%) and more diverse categories of AS affected genes than the Ascomycota (AS rate 7.0%, excluding yeasts). Contrarily, AS is nearly absent in strict yeasts. We hypothesize that AS is rather common in many fungi and could facilitate mycelial and thallic complexity.

PR1.6
The redox-active fungal metabolite, gliotoxin, induces transcriptional remodelling in *Saccharomyces cerevisiae*.
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The opportunistic pathogen *Aspergillus fumigatus* is the cause of invasive aspergillosis and has a significant impact on mortality of immunocomprised patients. The redox active, non-ribosomal peptide, gliotoxin, which is highly toxic towards animal cells and fungi is produced by this organism however the precise mechanism of its anti-fungal activity remains to be elucidated. We have identified and characterised proteins and genes that are up- or down-regulated in *A. fumigatus* and *Saccharomyces cerevisiae* in response to gliotoxin exposure. Proteomic analysis of *A. fumigatus* exposed to gliotoxin (14 micro g/ml) revealed *de novo* expression of a short chain dehydrogenase, eukaryotic translation elongation factor β1 and Cu/Zn super oxide dismutase; as well as a reduction in the expression of the mycelial catalase AFUA_6G10660 was observed.

To assess the effects on global gene expression in *Saccharomyces cerevisiae*following exposure to gliotoxin we applied RNAseq technology. Exposure to 16 or 64 µg/ml gliotoxin caused up- and down-regulation of genes involved in sulphur and carbohydrate metabolism and oxidative stress resistance.

A candidate gene approach identified *S. cerevisiae* mutants that exhibited altered sensitivity to gliotoxin exposure. Strains were chosen, based upon proteomic data which identified proteins showing induction/repression in *A. fumigatus* following exposure to gliotoxin. In contrast to a previous study that screened a *S. cerevisiae* gene deletions, we identified that deletion of the γ-glutamylcysteine synthase 1 gene (*GSH1*), confered resistance to gliotoxin (16 µg/ml) compared to wild type yeast strains. Also, mutants which lacked either the Cu/Zn superoxide dismutase gene (*SOD1*) or the Yeast activating protein (*YAP1*) gene resulted in hypersensitivity in the presence of gliotoxin compared to wild type strain. No growth difference was observed when using Δ*CTT1*, Δ*GSH2* or Δ*GLR1.*

Our data indicates that exposure to gliotoxin causes a complex *in vivo* transcriptional remodelling altering the expression of genes involved in metabolic pathways, while also appearing to induce oxidative stress in fungal cells.

PR1.7

Exploring the early symptomless phase of *Fusarium graminearum* infection of wheat ears

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Globally, *Fusarium graminearum* infections reduce cereal grain yield and contaminate grain with harmful trichothecene mycotoxins. *F. graminearum* forms an intimate intercellular association with the host species wheat whilst infecting the floral tissues (1). *TRI* gene expression required for deoxynivalenol (DON) mycotoxin production is maximal at the advancing hyphal front (2). This finding supports the hypothesis that DON plays a role in inhibiting plant defences. During this latent infection period, extracellular communication between live pathogen and host cells must occur, implying a role for secreted fungal proteins. In the current study, a refined *F. graminearum* secretome was predicted by combining several bioinformatic approaches (3). A comprehensive comparative genomic analysis involving 57 fungal and oomycete genomes revealed that very few predicted *F. graminearum* secreted proteins are species specific (3). A simplified linear system of rachis infection has now been used in conjunction with next generation sequencing (Illumina) and Affymetrix array technologies to explore globally the repertoire of *F. graminearum* genes specifically expressed at the intercellularly advancing hyphal front. To investigate gene function we are using the Barley Stripe Mosaic Virus vector to express transiently *in planta* sequences coding for secreted proteins of most interest.

1. Brown et al. (2010) Fungal Biology 114, 555-571.
2. Brown et al. (2011) Journal of Pathogens, Article ID 626345
3. Brown et al. (2012) PLoS One (in press)

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PR1.8

The Num1 protein in *Ustilago maydis* – a novel connection between splicing and transport

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In the corn smut fungus *Ustilago maydis*, sexual development is initiated by the fusion of two haploid sporidia, resulting in a filamentous dikaryon that is capable to infect the plant. Growth as a dikaryon requires an elaborate coordination of the cell cycle, the migration of the nuclei and polar hyphal growth. We have identified the Num1 protein with a pivotal function during these processes. Num1 is homologous to SPF27, a core component of the evolutionary conserved Prp19/CDC5 complex, an integral component of active spliceosomes. In addition to regulating spliceosome formation and splicing fidelity, the complex is involved in DNA damage repair and cell cycle checkpoint control. Hyphae of *num1* deletion strains exhibit pleiotropic polarity defects and a decreased splicing efficiency was verified upon realtime PCR analyses; additionally, the *num1* mutation affects the cell cycle and cell division as well as survival upon UV-irradiation. Using the Yeast-Two-Hybrid system and Co-immunoprecipitation analyses, we identified Cdc5 and Prp19, two further components of the Prp19/CDC5 complex, as Num1 interactors. Moreover, we identified proteins with functions during vesicle-mediated transport processes; in particular the kinesin 1 motor protein Kin1 was shown to physically interact with Num1. Both *num1* and *kin1* deletion strains exhibit identical phenotypes, corroborating their genetic interaction. As our data connect the splicing machinery and long distant transport we performed an mRNA-Seq experiment to elucidate which specific transcripts are affected for splicing in the *Δnum1* mutant on a global level. We will present our current view how these two disparate mechanisms may be linked.

PR1.9

The *N. crassa* Bem46 protein is involved in ascospore germination and interacts with the F domain of anthranylate synthase component II

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The bud emergence (BEM) 46 proteins are evolutionarily conserved members of the *α/β*-hydrolase super family. The exact function(s) of the protein remain unknown. Vegetative hyphae, perithecia and ascospores of *Neurospora crassa* RNAi and over-expressing transformants develop normally, but hyphal germination from ascospores is impaired. These results indicate a role of BEM46 in maintaining cell type-specific polarity in *N. crassa*. The protein is localized in the perinuclear endoplasmatic reticulum and also forms spots near to the plasma membrane (Mercker et al. 2009). The use of Lifeact-TagRFP (Lichius & Read pers. comm.) and Bem46-eGFP in heterokaryons of *N. crassa* indicated that the Bem46 protein is not interacting with actin. Likewise, the use of the lipid raft-stainer TexasRed™ showed no co-localization with Bem46-eGFP. We currently analyze the potential co-localization of Bem46 with the eisosomal protein LSP1. To that end we cloned the corresponding *N. crassa* ortholog of *lsp1* and fused it to RFP.

A yeast two-hybrid approach was undertaken using a previously established *N. crassa* two-hybrid library (Seiler pers. comm.). We identified one interacting protein, the anthranylate synthase component II (Walker & DeMoss 1986). Further investigation showed that the BEM46 protein is likely to interact with the F domain of that protein, which is a N-(5'-phosphoribosyl) anthranylate isomerase. The interaction was confirmed *in vivo* by employing bimolecular fluorescence complementation assays.

References:

Mercker M, Kollath-Leiß K, Allgaier S, Weiland N, Kempken F (2009) Curr Genet 55:151-161
Margaret S. Walker & John A. DeMoss (1986) J Biol Chem 261:16073-16077

PR1.10

***Trichoderma* glutamate decarboxylase plays an important role during germination and hyphae development**

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Glutamate decarboxylase (GAD) catalyzes glutamate decarboxylation to gamma-aminobutyrate (GABA) and is the first enzyme of the GABA shunt cycle. Endogenous changes in GABA levels in fungi correspond with developmental changes during conidiation and/or germination. It was observed previously that GAD activity in *Trichoderma atroviride* F-534 changes during conidiation, early germination and stationary phase of submerged culture. A lack of GAD activity during batch culture on sucrose as a sole carbon source lead to several aberrations in morphology and development: increased formation of chlamydospore-like structures, longer lag-phase, lower germination rate, higher number of bipolar germ tubes and increased hyphae branching. It has been demonstrated before that in vitro analysis of GAD activity in *Trichoderma* decreased in presence of cyclosporine A, a calcineurin signaling pathway inhibitor. Wild type strain in cultures containing various concentrations of cyclosporine A exhibited similar phenotypic characteristics of the delta *gad* strains – increased bipolar germination and hyphae branching. The similar effects of cyclosporine A have been known in other filamentous fungi (*Neurospora, Aspergillus*). Bipolar germination and branching of *delta gad* strains remained unaffected in the presence of cyclosporine A indicating that GAD could function as a downstream effector of calcineurin.

PR1.11

Discovery of a novel peroxisomal function for biotin biosynthesis in *Aspergillus oryzae*

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Peroxisomes, ubiquitous organelles in eukaryotic cells, typically contain enzymes involved in β-oxidation of fatty acids, and play diverse roles in different eukaryotic organisms. In filamentous fungi, for example, they are required for the formation of Woronin body that is an organelle specific to filamentous ascomycetes and functions in the septal pore plugging upon hyphal injury. Furthermore, peroxisomes are reported to play fundamental roles during growth in humans, plants and also filamentous fungi. However, molecular mechanisms responsible for the severe growth defects of peroxisomal deficiency remain unknown.

Here we generated the *Aspergillus oryzae* strains with deleted *Aopex5* and *Aopex7* genes encoding the receptors for peroxisomal targeting signals PTS1 and PTS2, respectively. In addition to their growth defects in the minimal medium containing oleic acid as a carbon source, surprisingly, they exhibited growth defects on the glucose medium. By screening for required nutrient supplements, we found that the addition of biotin restores their growth defects. Genome database searches revealed that BioF protein/KAPA (7-keto-8-aminopelargonic acid) synthase, one of the biotin biosynthetic enzymes, has a PTS1 sequence. Both Δ*AobioF* and *AobioF*Δ*PTS1* strains exhibited growth defects in the absence of biotin, indicating that peroxisomal targeting of BioF is crucial for the biotin biosynthesis.

In conclusion we demonstrate a novel role for peroxisomes in biotin biosynthesis, of which steps are upstream of the terminal reactions in mitochondria.

PR1.12

Endogenous short RNAs generated by Dicer-2 and RNA-dependent RNA polymerase-1 regulate mRNAs in *Mucor circinelloides*

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Endogenous short RNAs (esRNAs) play diverse roles in eukaryotes including viral defence, transposon silencing, heterochromatin formation and post-transcriptional silencing of protein coding genes. Most esRNAs are produced from double stranded RNA (dsRNA) by Dicer and loaded into a RISC complex containing an Argonaute protein. esRNAs are grouped into different classes based on biogenesis and function but not all classes are present in all eukaryotic kingdoms. It is not clear what esRNA classes are present in fungi and whether they regulate the expression of protein coding genes. By deep sequencing of esRNAs in the wild type and silencing mutants we have shown that the opportunistic pathogen *Mucor circinelloides* does not contain microRNAs but produces new classes of esRNAs, which map to exons and regulate the expression of protein coding genes. The main class of these exonic-siRNAs (ex-siRNAs) is generated by RNA-dependent RNA Polymerase-1 (RdRP-1) and Dicer-like-2 (Dcl-2), although there is some redundancy between RdRP-1/RdRP-2 and Dcl-1/Dcl-2 proteins. Ex-siRNAs target the mRNAs of protein coding genes from which they were produced and their accumulation requires the function of the *argonaute-1* (*ago-1*) gene, which suggests that binding of ex-siRNAs to Ago-1 is required for their stabilization. Genes regulated by this class of ex-siRNAs are mainly involved in signal transduction and information storage and processing. Other classes of ex-siRNAs require different combination of Dicer and RdRP proteins for their biogenesis and target different set of genes. Our results expand the range of esRNAs in eukaryotes and reveal a new role for esRNAs in fungi.

R1.13

Microtubule-dependent co-transport OF mRNPs and endosomes in *Ustilago maydis*

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Long-distance transport of mRNAs is important in determining polarity in eukaryotes. In *U. maydis* this process is mediated by the RNA binding protein Rrm4 which is a key component of large motile ribonucleoprotein complexes (mRNPs) shuttling along the microtubule cytoskeleton. Deletion of *rrm4* disrupts long-distant transport of mRNP and leads to defects in filamentous growth and a reduced virulence. Here we show that the plus end-directed UNC104/Kif1A-like Kinesin 3 as well as the minus end-directed split dynein Dyn1/2 are involved in the shuttling of the Rrm4-containing mRNPs. Kin3 transports the mRNPs to the poles of the growing hyphae, whereas Dyn1/2 mediates the retrograde movement of the mRNPs. Furthermore we demonstrate co-localisation of Rrm4-containing mRNPs with the t-SNARE Yup1 on endosomes, that are transported by the same set of motors. Interfering with endosome function, loss of Kin3 or removal of its lipid-binding pleckstrin homology domain abolishes Rrm4-dependent movement without preventing co-localisation of Rrm4 and Yup1-positive endosomes. This data suggests vesicle hitchhiking as new mechanism of mRNP transport.

PR1.14

Export of staurosporine and glutathione by Neurospora crassa

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ATP-binding cassete (ABC) transporters are membrane proteins that use the energy of ATP hydrolysis to drive the transport of substrates across membranes. The death-inducer drug staurosporine (STS) up-regulates the expression of the ABC transporter protein ABC-3 in Neurospora crassa, as revealed by microarrays, qPCR and western-blotting. Taking advantage of STS fluorescence, a flow cytometry methodology was devised to measure STS intracellular content. The abc-3 deletion mutant revealed to be deficient in STS export, resulting in high intracellular accumulation of the drug, and pointing to a role of the ABC-3 protein as a STS exporter. Accordingly, the mutant was found to be highly sensitive to STS-induced cell death, as revealed by impaired germination and increased DNA fragmentation, and classical inhibitors of ABC transporters enhanced the effects of STS. We also found that STS-induced cell death in N. crassa is accompanied by the export of reduced glutathione (GSH). Surprisingly, the abc-3 null mutant was found to be deficient in STS-induced glutathione export, suggesting an additional role for the ABC-3 protein in the transport of GSH. The hypothesis of a co-transport of STS and GSH by the N. crassa ABC-3 protein is currently under investigation.

PR1.15

Lipidomic-based approach to identify possible lipid compounds able to modify *Fusarium verticillioides* metabolism

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Fusarium verticillioides is one of the most important fungal pathogen to cause ear and stalk rot in maize, even if frequently asymptomatic. The interest for this fungus increased significantly after the discovery of fumonisins, hypothesized to contribute to *F. verticillioides* colonization and confirmed harmful to human and animal health. The understanding of the mechanisms associated with pathogenicity and fumonisin biosynthesis in *F. verticillioides* could help to mitigate *Fusarium* diseases.

Endogenous fungal oxylipins are known for their roles in carrying out pathogenic strategies to successfully colonize their host, reproduce, and synthesize toxins.

A synthetic medium (CDY), amended (FB_1-conducive) or not (FB_1-non conducive) with lyophilized maize, inoculated with *F. verticillioides* has been used for studying the importance of the lipid by-products such as oxylipins and their role in fumonisin B_1 (FB_1) formation. We analysed some molecular and physiological parameters: the expression of genes whose products are related to oxylipin synthesis (such as lipoxygenase, diol synthases and fatty acid oxidases) by relative Real Time PCR, the fatty acids and oxylipin profile using a lipidomic approach, i.e. combining LC-TOF with a robust statistical analysis (i.e. PCA) and the FB_1 biosynthesis by HPLC.

The results obtained indicate that the presence of lyophilised maize induces the up-regulation of all the genes analysed, the modification of lipid profile of *F. verticillioides* and the biosynthesis of FB_1 without significantly affecting fungal growth. The crucial role of lipid compounds in *F. verticillioides* lifestyle emerges also in this study.
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PR1.16

Vegetative hyphal fusion in epichloae endophytic fungi

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Vegetative hyphal fusion has been hypothesized to play an important role in the emergence of fungal diversity, including the generation of interspecies hybrids. The ability to form fused hyphae also appears to be related to fungal sexual reproduction, since genes involved in the former are often required for the latter as well. Here, we used epichloae endophytic fungi as models to study the potential correlation between vegetative hyphal fusion and fungal diversity, since they include both sexual (*Epichloë*) and asexual (*Neotyphodium*) species, with the latter consisting of interspecies hybrids and non-hybrids. Subcellular staining and confocal laser scanning microscopy revealed that the representative *Epichloë festucae* isolate Fl-1 forms complete hyphal fusion with a continuous cytoplasm being established. It also formed hyphal fusion during endophytic growth in a tall fescue plant. Calcofluor White staining and DIC optics revealed that most *Epichloë* isolates form vegetative hyphal fusion in cultures. A majority of *Neotyphodium* isolates were also capable of hyphal fusion; however, a substantial portion of non-hybrid *Neotyphodium* species appear to lack this ability. Our data favor the hypothesis that the ability to undergo hyphal fusion is important for interspecies hybrid formation, and support its importance in the emergence of fungal diversity.

PR1.17

The role of *Aspergillus nidulans* myosin V, MyoE, in hyphal tip growth

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Aspergillus nidulans has a single myosin V heavy chain gene which we designate *myoE*. *myoE* is not essential but deletion of *myoE* causes abnormal hyphal morphology and branching anterior to the first septum. Tip extension in *myoE* deletants is slower than in controls, but because the hyphal diameter is greater, the growth rate (increase in volume/ unit of time) is only slightly reduced relative to myoE^{+} controls. Growth is extremely slow in the absence of MyoE and microtubules, but not completely inhibited. MyoE-GFP localizes to the Spitzenkörper (a vesicle supply center) and to puncta in the cytoplasm that move bidirectionally. Treatments with inhibitors show that the localization of MyoE at the Spitzenkörper is actin dependent but movement of some of the MyoE puncta in the hyphae is actin independent. Time-lapse imaging of SynA, a v-SNARE, reveals that in *myoEΔ* cells vesicles no longer localize to the Spitzenkörper. Photobleaching experiments with GFP-SynA show that in myoE^{+} cells, the SynA exocytic vesicles move rapidly from the non-bleached area of the hypha to the Spitzenkörper before incorporation into the plasma membrane, whereas in *myoEΔ* cells they do not localize to the Spizenkörper. Rather they move directly to the plasma membrane. Our data indicate strongly that MyoE function is required for the accumulation of vesicles at the Spitzenköper which facilitates faster tip extension and a normal hyphal shape.

PR1.18

MAPK pathways are key regulators of the chemotropic response in *Fusarium oxysporum*

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Fungal hyphae have the capacity to explore the surrounding environment and to grow towards gradients of tropically active cues. Chemotropic responses to nutrients, sex pheromones or host compounds play a crucial role in fungal development and virulence, but the underlying mechanisms remain poorly understood. We used a genetic approach to dissect chemotropism in the soilborne vascular wilt pathogen *Fusarium oxysporum*. A quantitative assay was used to measure directed growth of germ tubes towards different classes of compounds, including carbon and nitrogen sources, sex pheromones, plant secondary metabolites and root exudates. Mutants lacking the mitogen activated protein kinase (MAPK) Fmk1 or the transcription factor Ste12, two components of the conserved Pathogenicity MAPK cascade, were impaired in chemotropism towards nutrients, but fully responsive to α-pheromone and tomato root exudates. By contrast, Rho1 and Mpk1, two components of the cell integrity MAPK cascade, were specifically required for directed growth towards root exudates or α-pheromone. We further identified several transmembrane proteins involved in chemoattractant sensing, including the G-protein coupled receptor Ste2, the signalling mucin Msb2 and the tetraspan adaptor protein Sho1. Our results suggest that distinct MAPK signalling pathways mediate chemotropism of *F. oxysporum* towards nutrients and pheromone-like compounds.

PR1.19

Functional analysis of glycoside hydrolase family 18 and 20 genes in *Neurospora crassa*

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Glycoside hydrolase (GH) family 18 and 20 contain chitinolytic (chitinase and NAGase respectively) enzymes responsible for chitin degradation during various aspects of fungal biology. GH18 are phylogenetically divided into 3 groups (A, B and C), each further divided into subgroups.

Subgroup B5 genes encodes enzymes with ENGase deglycosylation activity. In this study,we investigated the functional role of 10 *N.crassa* genes coding for chitinases, 2 genes encoding ENGases and 1 gene encoding a NAGase, using gene disruption and qPCR techniques. The ENGasedisruption mutant Δ*gh18-10* showed slower growth rate on carbon rich media and on chitin plates when compared with the wildtype (WT), while it grew faster than WT during abiotic stress conditions. *gh18-10* was constitutively expressed during growth on carbon rich media, during carbon starvation conditions, on chitin plates and during fungal–fungal interactions. The function of gh18-10 may be connection with the endoplasmatic reticulum associated protein degradation process (ERAD), a stringent quality-control of protein folding. Furthermore, the two C2 subgroup chitinase genes *gh18-6* and *gh18-8* were both induced during fungal–fungal interactions. However, *gh18-6* was only induced during interspecific interactions, while *gh18-8* displayed the highest expression levels during self–self interactions. *gh18-8* also displayed a unique domain structure including 2 transmembrane domains, indicative of cell wall localization. These data suggest functional differentiation of *N. crassa* C2 chitinases; gh18-6 may function in aggressive interspecific interactions while gh18-8 may be a cell wall modifying enzyme.

PR1.20

Two functionally distinct RNA-dependent RNA Polymerases participate in the Dicer-independent degradation of endogenous mRNAs in *Mucor circinelloides*

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RNA-dependent RNA polymerases (RdRPs) participate in the initiation and/or amplification steps of the RNA silencing mechanism in different organisms. The role of these enzymes is not limited to silence exogenous sequences but they are required for production of endogenous siRNAs that mainly map to repetitive sequences and transposons. We have tested the role of two different *rdrp* genes in exogenous and endogenous silencing in the zygomycete *Mucor circinelloides*, a basal fungus evolutionary distant from other fungal model organisms. Analysis of *rdrp*$^{-}$ disruption mutants indicates a functional diversification of both genes in transgene-induced silencing. Gene *rdrp-1* participates in the initiation of silencing by sense transgenes by producing antisense transcripts derived from the transgenes, but it does not have a role in dsRNA-induced silencing. On the other hand, *rdrp-2* is required for efficient amplification of silencing and accumulation of siRNAs regardless the nature of the silencing trigger. Differences in the phenotype of mutants of each gene highlight the cellular and physiological relevance of the functional diversification. Small RNA analyses in the wild-type strain and mutants affected in silencing genes demonstrate that both *rdrp* genes are involved in a novel degradation process of endogenous mRNAs that is *dicer*-independent. This new regulatory pathway, which does not include discrete sRNA molecules, mainly regulates the level of expression of housekeeping genes by specific degradation of mRNAs. Our results expand the role of RdRPs in gene silencing and reveal a new RNA degradation process that could represent the initial step in the evolution of RNA silencing.

PR1.21

Two Highly Conserved Arginines at the N-terminus of the NDR Kinase COT1 are Required for its Proper Function Via Interactions with MOB2A/2B

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Nuclear Dbf2-related (NDR) kinases are important for growth and development in *Neurospora crassa* and other filamentous fungi, and require the physical interaction with MPS1-binding (MOB) proteins for proper activity and function. Dysfunction of the *N. crassa* NDR kinase COT1 leads to cessation of tip extension and hyperbranching. In order to further characterize COT1/MOB interactions, we mutated two highly conserved Arg residues (R167 and R203) at the N-terminal region of COT1 (the MOB-binding domain) to Ala and inserted these alleles at the *cot-1* locus. Both point-mutated *cot-1* strains exhibit, at different extents, abnormal hyphal morphology, typified by hyphal swelling, dichotomous branching and cytoplasmic leakage along the aerial hyphae. In addition, these mutants produce less macroconidia. In spite of the mentioned defects, general colony morphology and growth rate of the mutants was only slightly affected. Interestingly, the hyphal and conidiation defects of both arginine mutants were suppressed in a Δ*mob-2b* background. In contrast, the Δ*mob-2a;cot-1*^{R203A} strain grew as a small fluffy colony that produced massive amounts of aerial hyphae with delayed carotenoid biosynthesis. Hyphae in this strain were slow growing, highly dense and hyper-branching was evident. These results establish the involvement of R167 and R203 in the regulation of COT1 function and in the interactions between COT1 and MOB2A/2B. As we have determined the occurrence of a physical interaction between the Arg methyl transferase SKB1 and COT1, it is conceivable that Arg methylation has a role in regulation of COT1 function.

PR1.22

High temperature growth arrest in of *Cryptococcus neoformans* mediated by APE4 an aspartyl amino peptidase involved in authophagy.

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Cryptococcus neoformans is an opportunistic pathogen that can cause fungal meningitis in immune compromised patients. The treatment choices for cryptococcosis are limited and resistant strains have being reported. However, in order to develop new drugs against this mycosis it is necessary to broad the knowledge about this yeast biology. Its ability to grow at high temperature is an important virulence factor and a key feature during pathogenesis. Therefore, we have screened a mutant collection generated by *Agrobacterium* random insertional mutagenesis aiming to uncover new genes that may be involved in high temperature growth. The inactivation and deletion of *APE4*, an aspartyl amino peptidase which localizes to degradation vesicles and is involved in autophagy in *S. cerevisiae*, leads to growth arrest at 37°C. We will present the role of this gene on the virulence and biology of *Cryptococcus neoformans*.

PR1.23

The role of *Cryptococcus neoformans* URA4 on de novo pirimidine biosynthesis pathway and its impact upon high temperature growth.

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Cryptococcus neoformans is an opportunistic fungus that has the ability to infect immune compromised individuals causing mild respiratory infection, pneumonia and meningitis, leading to the death if untreated. The increase of the immune compromised population and isolation of strains resistant to the treatment the knowledge about the biology of this organism, as well as its virulence factors, became desirable. Among its virulence factors, special attention has been dedicated to high temperature growth which is a key feature during pathogenesis. We have created and screened a library generated by *Agrobacterium* random insertional mutagenesis aiming to uncover new genes that may be involved in high temperature growth that could also be a new target for antifungal therapy. We found that the inactivation and deletion of *URA4* gene leads to growth arrest at 37º C. *URA4,* codes for a dihydroorotase involved in the conversion of carbamoyl aspartate to dihydroorotate in the *de novo* pyrimidine biosynthesis pathway. We will present the role of this gene on the virulence and biology of *C. neoformans*.

PR1.24

Functional characterization of *Aspergillus nidulans* RpdA: identification of complex partners and subcellular localization

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In eukaryotic organisms DNA is compacted into an elaborate structure called chromatin, thus enabling regulation of transcription by controlling the accessibility of the genetic information for transcription factors. Among the key players involved in the regulation of chromatin structure are histone acetyltransferases and histone deacetylases (HDACs)– enzymes establishing distinct acetylation patterns in the N-terminal tails of core histones. In filamentous fungi only little is known about the biological functions of these enzymes; nevertheless recent studies have shown that class 2 HDACs affect the regulation of genes involved in stress response and secondary metabolite production. Depletion of RpdA, a class 1 HDAC of *Aspergillus nidulans*, leads to a drastic reduction of growth and sporulation. Functional studies revealed that a short C-terminal motif unique for RpdA-type proteins of filamentous fungi is required for catalytic activity and consequently cannot be deleted without affecting the viability of *A. nidulans.* In order to further characterize RpdA we have started to analyze complex formation and localization of the protein with respect to this motif by expressing TAP- and GFP-tagged RpdA versions. First results indicate that both tagged full-length proteins are functional and suggest a role of the C-terminal motif for proper subcellular localization.

PR1.25

The impact of ornithine and arginine biosynthesis on siderophore production of *Aspergillus fumigatus*

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The opportunistic fungal pathogen *Aspergillus fumigatus* produces extracellular siderophores for iron uptake and intracellular siderophores for storage and distribution of iron. Moreover, *A. fumigatus* employs a second high-affinity iron acquisition system, reductive iron assimilation (RIA). Siderophore biosynthesis (SB) but not RIA is essential for virulence. The main precursor of siderophores, ornithine, can be produced from glutamate in the mitochondria or cytosolic hydrolysis of ornithine-derived arginine.

Here, the impact of inactivation of mitochondrial ornithine biosynthesis (*ΔargEF* mutant lacking N-acetylglutamate kinase/ N-acetylglutamylphosphate reductase) and cytosolic arginine biosynthesis (*ΔargB* mutant lacking ornithine transcarbamoyl transferase) on siderophore production was studied. Both *ΔargEF* and Δ*argB* are arginine auxotrophic. Growth of *ΔargEF* but not *ΔargB* is partially rescued by ornithine supplementation. Blocking RIA by ferrous iron chelation inhibited growth of *ΔargEF* but not *ΔargB.* Siderophore production of *ΔargEF* decreased while that of *ΔargB* increased with declining arginine availability. Taken together, these data indicate that the siderophore system is mainly fueled by mitochondrial rather than cytosolic ornithine production and that mitochondrial ornithine biosynthesis is feedback inhibited by arginine. In agreement with the SB defect, *ΔargEF* displayed a dramatically reduced cellular ornithine content. In contrast, the arginine and polyamine contents were wild type-like, indicating prioritization of the later two biosynthetic pathways over SB. Consistent with cellular balancing of SB and arginine metabolism, arginine was recently identified to allosterically activate the ornithine monooxygenase SidA and consequently SB-mediated ornithine consumption.

PR1.26

Highly conserved signalling multi subunit protein complexes in the filamentous fungus *Sordaria macrospora*

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The homothallic ascomycet *Sordaria macrospora* can be easily applied as a model organism for research in cellular development. From a set of sterile mutants showing only protoperithecia formation, we were able to characterise the PRO-proteins involved in sexual development. The characterisation of three PRO proteins led to the identification of two different conserved multi subunit complexes involved in signalling during cellular differentiation [1, 2, 3]. PRO40, the homolog of Soft from *Neurospora crassa*, supposedly works as a scaffold in the PRO40-MAPK interaction network conserved in ascomycets. Besides the MAPK cascade kinases and PRO40, the multifunctional protein SPG20 is involved in this complex. For the first time in filamentous fungi the highly conserved striatin interacting phosphatase and kinase (STRIPAK) was characterised in *S. macrospora* via different approaches [4]. PRO11 is the homolog of human striatin and probably is a scaffolding subunit in this complex [1]. PRO22 is also highly conserved among eukaryotes and is homologous to human STRIP ("striatin interacting protein") [3]. As part of the STRIPAK complex, it was possible to show the interaction of PRO22 and the scaffolding subunit PP2AA of the heterotrimeric protein phosphatase PP2A [4]. These two multi modular protein complexes play a crucial rule in signalling during the transition from vegetative growth to sexual development and are possibly linked through scaffolding or anchoring proteins to a single large and highly conserved signalling multi subunit protein complex.

[1] Pöggeler and Kück (2004), Eukaryot Cel, [2] Engh et al. (2007), Eukaryot Cell
[3] Bloemendal et al. (2010), Eukaryot Cel, [4] Bloemendal et al. (2011), in prep.

PR1.27

In-Depth analysis of Pectin Degradation and Catabolism by the Model Filamentous Fungus *Neurospora crassa*

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Pectin, e.g. isolated from apple pulp or citrus peel, is a well-known commodity of the food and medical industry. Although in varying amounts, Pectin is present in all plant biomass, being the main constituent of the middle lamella, which is the cell wall layer connecting adjacent cells.

As such, it is also a source of fermentable sugars in the production of biofuels or value-added by-products from plant-based feedstocks. Our institute is using *Neurospora crassa* to study the enzymatic depolymerization of plant cell walls by filamentous fungi, as it is not only an efficient cell wall degrader, but also a well-established model system. In the present study we are analyzing the "toolbox" that *Neurospora* unfolds when growing on Pectin as its sole carbon source. Using a combination of shotgun proteomics and functional genomics we are trying to identify the Pectin-specific secretome as well as all the genes involved in the subsequent catabolism of the resulting sugars. *Neurospora* was found to secrete more than 70 proteins on Pectin and orange peel from at least 25 carbohydrate-active families. RNA sequencing allowed us to screen the entire genome for genes specifically induced by Pectin. Furthermore, strains containing deletions in genes (potentially) involved in Pectin catabolism were analyzed for their phenotype when grown on pectin and any difference in their rate of pectin consumption in comparison to the WT. Our results demonstrate that *Neurospora*, with its well-established genomics resources in combination with proteomic tools, is ideally suited to characterize fungal polysaccharide degradation in unparalleled detail.

PR1.28

Fungal siderophore excretion is mediated by a new fungal-specific subfamily of ABC transporters

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The opportunistic human pathogen *Aspergillus fumigatus* produces extra- and intracellular siderophores for acquisition, storage and intracellular distribution of iron. The siderophore system is essential for virulence and therefore represents an attractive antifungal target. Here we report the functional characterization of three ABC transporters, termed AbcB, AbcC, and AbcD. All three transporters are transcriptionally repressed by iron via the GATA-factor SreA. Moreover, the genes encoding AbcB and AbcC are located within siderophore metabolic gene clusters indicating a function in siderophore metabolism. Enhanced green fluorescent protein (EGFP)-tagging localized AbcB in the plasma membrane. Inactivation of AbcB blocked excretion of the siderophore fusarinine C (FsC) and increased intracellular accumulation of FsC degradation products. Deletion of AbcC decreased excretion of triacetylfusarinine C (TAFC) but increased excretion of its precursor FsC. Inactivation of AbcD reduced siderophore excretion only in AbcB- or AbcC-deficient backgrounds, implying mutual compensation of these ABC-transporters. Inactivation of all three ABC transporters completely blocked siderophore excretion. Consistently, inactivation of AbcB, AbcC, either two of the three, and particularly all three ABC transporters impaired growth during iron starvation but not iron sufficiency. Phylogenetic analysis revealed that AbcB, AbcC and AbcD are members of a new subfamily of the ABC transporter superfamily conserved in all siderophore-producing but not siderophore-lacking fungal species, indicating involvement of all subfamily members in siderophore metabolism.

PR1.29

Identification and Characterization of a Novel Secondary Metabolite Gene Cluster in *Aspergillus nidulans.*

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Fungal secondary metabolites are a rich source of medically useful compounds due to their pharmaceutical and toxic properties. Sequencing of fungal genomes has revealed numerous secondary metabolite gene clusters, yet products of many of these biosynthetic pathways are unknown since the expression of the clustered genes usually remains silent under normal laboratory conditions. We discovered a novel secondary metabolite in *Aspergillus nidulans* by predicting a biosynthetic gene cluster with genomic mining. We identified a Zn(II)2Cys6–type transcription factor and demonstrated its role as a pathway-specific activator for the cluster genes. Quantitative real-time PCR and DNA array analysis showed that overexpression of the putative transcription factor leads to upregulation of seven genes in the predicted cluster area. The GC/MS analysis of the strain with an activated gene cluster revealed production of a novel secondary metabolite for *Aspergillus nidulans*. In addition, we noted an increase in sexual fruiting body formation and a reduction in conidiation in the metabolite producing strain. The change in the fungal morphology seen in the overexpression strain could indicate a link between development and activated secondary metabolism.

PR1.30

Protein map of cytosolic proteins of the Agaricomycete *Schizophyllum commune*

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The white-rot fungus *Schizophyllum commune* is a model organism for microbiological and molecularbiological studies. Since the genome of *S. commune* has been published, it is easy to perform studies not only on the genome but also on the proteome of this fungus. Therefore, we prepared 2 dimensional protein gels with a pH range from 3 to 11 for cytosolic proteins of the monokaryotic strain 12-43, which acts as the reference strain. More than 700 protein spots were detected on the protein gel and 527 were chosen for mass spectrometry analysis *via* MALDI-TOF.

The reference strain was compared to a second monokaryotic strain 4-39 and a dikaryotic strain, which demonstrates the main mode of life of *S. commune*. Several differences could be ascertained. There are additional proteins in the monokaryotic and dikaryotic strain as well as differences in the expression of proteins compared to the mastergel of strain 12-43. In contrast, a few proteins are missing in the comparison specimen.

With this study we will simplify proteome comparisons of wildtypes and mutants of *S. commune* by publishing the mastergel as protein map with detailed information of the analyzed protein spots.

PR1.31

The role of calmodulin during cell fusion and colony initiation in *Neurospora crassa*

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Calcium signalling is involved in regulating many important processes in filamentous fungi including spore germination, hyphal growth, mechanosensing, stress responses, the cell cycle and circadian rhythms. As the primary intracellular Ca^{2+} receptor, calmodulin (CaM) converts Ca^{2+} signals into enzymatic signals by regulating the activity of numerous target proteins. We have found that both Ca^{2+}-free medium and two CaM antagonists (calmidazolium and trifluoperazine) selectively inhibit conidial anastomosis tube (CAT) fusion during colony initiation in *Neurospora crassa.* These results indicate that Ca^{2+}/CaM signalling is probably involved in regulating this process. In order to further analyse the role of CaM in regulating cell fusion during colony initiation, we have been imaging GFP labelled CaM in living cells. For this purpose we use both the native promoter and P*ccg-1* promoter of *N. crassa* to express N-terminal or C-terminal GFP tagged CaM. In germ tubes and CATs, CaM localized as dynamic dots associated with the plasma membrane and within the cytoplasm. In germ tubes CaM also localized at developing septa (CATs lack septa). However, CATs that were undergoing chemoattraction towards each other showed a distinctly different pattern of localization at their growing tips; CAT tips showed a very pronounced accumulation of Cam whilst germ tube tips did not. We are currently co-localizing GFP labelled CaM with RFP labelled for nuclei, F-actin and microtubules to analyse their dynamic temporal and spatial relationships with each other.

PR1.32

Investigating the role of actin and microtubule cytoskeletons in endocytosis of plant pathogen *Ustilago Maydis*

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Endocytosis is essential for pathogenic development in *Ustilago maydis*. It has been shown that the initiation of pathogenicity relies upon the cell's ability to recognise the pheromone *(a1 or a2)* released from its mating partner and to form conjugation hyphae *(Urban et al., 1996b)*. In yeast the actin cytoskeleton is essential for endocytosis and the absence of actin function can cause blocked internalisation of endocytic cargoes *(Raths et al., 1993)*. We have produced a synthetic, fluorescently labelled pheromone marker and observed its localisation over time in *Ustilago maydis* cells. Our results strongly suggest the intra-cellular movement of the pheromone is driven by receptor-mediated endocytosis. Destabilisation of the cytoskeleton, using either benomyl or latrunculin A, inhibits the translocation of pheromone to the vacuole. Furthermore, we show the microtubule-dependent motors kinesin 3 and dynein are essential in the transport of the pheromone to the vacuole.

PR1.33

HYM1 functions as dual scaffold for NDR and MAP kinase pathways in *Neurospora crassa* to coordinate hyphal polarity with cell communication and sexual development

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HYM1/MO25-type proteins function as general co-activator proteins of germinal center kinases (GCKs), which activate nuclear-DBF2-related (NDR) kinase pathways and thereby regulate cellular morphogenesis and proliferation. Here we show that, in addition to a scaffold function of HYM1 for the POD6-COT1 GCK-NDR kinase complex in *N. crassa*, HYM1 is also critical for the NRC1-STE7-MAK2 MAP kinase pathway, which regulates vegetative cell-cell communication and sexual development. HYM1 interacts with all three kinases of the MAK2 MAP kinase cascade and co-localizes with MAK2 at the apex of growing cells. Deletion of *hym-1* phenocopies all defects observed for MAK2 pathway mutants by abolishing MAK2 activity. A NRC1-STE7 fusion protein reconstitutes MAK2 signaling, while constitutive activation of the individual MAPKKK and MAPKK proteins of the MAK2 pathway does not. These data identify HYM1 as novel scaffold for the NRC1-STE7-MAK2 pathway and establish HYM1 as central player for coordinating NDR and MAP kinase signaling during cell polarity, cell communication and sexual development.

PR1.34

The α-tubulin B-encoding gene and its expression in the phytopathogenic fungus Botrytis cinerea

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The plant pathogen fungus *Botrytis cinerea* is able to infect more than 250 hosts. It is responsible for major losses in crops, and grapes in particular in countries like France. The fungus life cycle relies on distinct developmental stages and specific cellular structures like appressoria, infection cushions, conidiophores and apothecia. Based on the important role that cytoskeleton plays in cellular organization and shape in all eucaryotes, attention was given to tubulins and their putative specific role in the virulence of *B. cinerea*. One β-tubulin and two α-tubulin encoding genes are found in *B. cinerea*'s genome. Phylogenetic analyses revealed that orthologs to the α-tubulin A gene exist in all asco- and basidiomycetous species whereas the α-tubulin B gene seems to be present in some ascomyceta only. In *B. cinerea*, tubulin A and B share 69% identity at the protein level, and the B gene is always more expressed than A. Besides, the expression of both genes follow a similar profile during growth and conidia-derived development. Interestingly, a peak of expression is observed early in development and expression is higher in infection cushions than in mycelium. To understand these changes in expression, transcriptional fusions and successive deletions were used to identify putative regulatory regions in the B gene promoter. In parallel, bioinformatic motifs searches were conducted to find putative DNA sequences involved in this regulation.

PR1.35

Interrogating the transcriptomes of developmental mutants to identify conidiation-specific genes in *Fusarium graminearum.*

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The vast majority of fungi propagate clonally through the formation of asexual spores (conidia). To produce conidia, fungi must undergo a defined program of morphological development, which presumably requires the coordinated efforts of many individual genes and signal transduction pathways. In the cereal pathogen, *Fusarium graminearum*, the morphological transition from filamentous growth to conidiation is critical for dissemination and infection. In spite of this, very little is known about the genetic regulation of this important developmental process. From genetic screens designed to identify genes regulating specific aspects of morphogenesis in *F. graminearum*, we identified several insertional mutants impaired in conidiation, which variously display a range of loss- and gain-of-function phenotypes. One mutant that fails to produce conidia, designated 8B5, contains an insertion within a putative bi-directional promoter of genes FG_10779 and FG_10780. To further understand the impact of the insertion on gene regulation, genome-wide analyses of gene expression were performed with microarrays [Fusarias520715 Affymetrix GeneChip] on the wild type and 8B5 mutant strain under conditions either favorable or unfavorable for asexual development. A total of 39 genes exhibited altered expression (>4 f.c.) in both wild-type and 8B5 under various culturing conditions. Analysis of these candidate genes revealed the presence of putative transcription factors (such as a white-collar homolog), structural genes (such as a chitin synthase homolog), five orphan genes, as well as two putative gene clusters. Additional findings stemming from our functional and comparative analyses of developmental mutants will be presented.

PR1.36

Identification Of Transcriptional Regulators Involved In Azole Resistance In The Pathogenic Fungus *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic human pathogen responsible for invasive and chronic disease. Treatment of fungal infections is becoming increasingly difficult due to the emergence of resistance particularly to the azole drug class. The key causes of clinical azole resistance are point mutations in the drug target (CYP51) however isolates with non-target resistance mechanisms are being frequently isolated in our patient population. In an attempt to identify some of these novel mechanisms we are exploring the role of transcription factors in itraconazole reistance.

Availability of the complete genome sequence of *A. fumigatus* has enabled us to identify 450 transcription factors. To enable disruption of all 450 of these genes we have devised a systematic approach to gene knockout coupling a 2-step PCR fusion approach to a 96-well transformation method. Our process, from the automated design of gene KO primers to generation of KO cassettes strains and subsequent isolation of mutant isolates takes less than one week per 96 strains. Several of the transcription factors we have identified appear to be critical to the viability of *A. fumigatus* whilst many other mutants exhibit a range of gross phenotypic effects. We are currently analysing our collection of gene deletion mutants to identify those transcription factors which are either resistant or sensitive to azoles and other antifungal agents.

PR1.37

The influence of the conditions of leaf extract on perithecia formation of *Pleospora herbarum*

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Many phytopathogenic fungi have both teleomorph and anamorph, and differentiate from hyphae depending on the conditions in the host plants. The fungi often lose the morphogenic ability and grow only in the hyphae form by the succession of in vitro culture. They, however, recover the ability if the hyphae could infect to the host plants. We have developed the method (ALP medium) that many fungi could form perithecia in vitro using lightly boiled gardenia and hydrangea leaves (Furukawa and Kishi 2000). These facts indicate that some common factors which induce perithecia formation in many fungi must exist in the leaves. We tried to extract the factors and re-investigate physical conditions, especially light wavelength, to induce perithecia. *Pleospora herbarum* (Pers.) Rabenh was used for the examinations. As a result, the activity existed in the oxidized aqueous fraction, which induced the perithecia and conidia abundantly under the dark condition. Fatty acids and the related compounds, which were reported to induce perithecia and conidia in *Aspergillus nidulans* (Calvo et al. 1999, Tsitsigiannis et al 2004, 2007), did not induce perithecia or conidia under light or dark conditions. Blue light, which is essential for conidia formation in *Neurospora crassa* (Olmendo et al. 2010), was not needed for *P. herbarum* on the medium containing oxidized leaf extract, though it was essential for perithecia production on other media. Those imply the morphogenic switch of phytopathogenic fungi is different from that of saprozonic fungi.

PR1.38

Subcellular localization of maltose permease (MalP) in response to carbon sources in *Aspergillus oryzae*

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A gene cluster (*MAL* cluster) involved in maltose utilization is also required for production of amylolytic enzymes in *Aspergillus oryzae*. In particular, maltose permease encoded by the gene *malP* in the *MAL* cluster is essential for uptake of maltose that induces the amylolytic genes through the activation of the transcription factor AmyR. The *malP* gene expression is induced by maltose and repressed by glucose [1]. However, it has not yet been examined how MalP is regulated at the protein level in response to carbon sources, although MalP is thought to be degraded through the endocytic pathway in the presence of glucose as MAL63 in *Saccharomyces cerevisiae*. In this study, we examined the effect of various sugar species on endocytosis of the MalP protein.

MalP fused to sGFP (MalP–GFP) localized at the plasma membrane, when expressed by own promoter in maltose medium. After addition of glucose, MalP–GFP was promptly internalized and delivered to the vacuole. This internalization was prevented by the addition of latrunculin B, an actin depolymerizing agent, indicating that MalP was targeted to the vacuole by endocytosis. Effect of the sugar species including glucose analogs on the internalization of MalP–GFP was investigated by fluorescence microscopy. Consequently, fructose, mannose, and 2-deoxyglucose as well as glucose triggered the internalization of MalP–GFP to the vacuole, whereas xylose, 6-deoxyglucose, and 3-*O*-methylglucose had no effect on MalP–GFP localization.

[1] Hasegawa et al. *Fungal Genet. Biol.* **47**, 1–9 (2010)

PR1.39

Establishment of tools for the analysis of vesicle traffic in *Aspergillus nidulans*

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In order to allow the extreme polar hyphal growth of filamentous fungi membrane lipids and cell wall synthesizing enzymes are needed constantly in the growth area. This is achieved by a continuous flow of secretory vesicles from the Golgi apparatus to the growing tip. For efficient growth, cell end markers and enzymes anchored in the plasma membrane are removed from the plasma membrane subapically by endocytosis. Thus filamentous growth depends on different populations of vesicles, which may use different routes for transportation. In order to investigate the dynamics and role of different vesicles, we studied the localization of different proteins of both the endocytotic and exocytotic pathway. SynA, a v-SNARE protein and clathrin heavy-chain are components of both pathways. In addition, three putative secreted proteins, β-glucosidase with a signal peptide (BglA) and two proteins without a signal peptide, the glucan synthase regulatory protein (GlsA) and the chitin synthase (ChsB), were studied.

By co-localization with fluorescently labeled microtubules we showed transport along these filaments. SynA, BglA, GlsA und ChsB were transported by conventional kinesin towards the Spitzenkörper, were they accumulated. After deletion of the kinesin-1 the anterograde transport was taken over by other motor proteins like kinesin-3.

At the tip the secretory vesicles containing SynA fused with the plasma membrane and were internalized at the subapical region. The internalized vesicles called early endosomes were transported by dynein.

PR1.40

Peroxisomal Localization Of Siderophore Biosynthesis In *Aspergillus* SSP.

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Virtually all organisms require iron as an essential nutrient. Siderophores, low molecular mass, iron-specific chelators, play a central role in iron acquisition, iron storage and virulence of various phyto- and animal-pathogenic fungi (1-3). However, the subcellular localization of siderophore biosynthesis is unknown. *Aspergillus fumigatus* and *Aspergillus nidulans* produce two major siderophores: extracellular triacetylfusarinine C (TAFC) for iron acquisition and intracellular ferricrocin (FC) for iron storage. Interestingly, two TAFC biosynthetic enzymes, SidH (*cis*-anhydromevalonyl CoA-hydratase) and SidF (N^5-hydroxyornithine:*cis*--anhydromevalonyl CoA-N^5 transacylase), possess putative peroxisomal targeting signals type 1 (PTS1), which are highly conserved in their orthologs of *Aspergillus ssp.* . Using N-terminal GFP-tagging of SidH and SidF, we could show that the TAFC biosynthesis is, in part, localized in peroxisomes. Addionally SidH and SidF were localized in peroxin mutant strains of *A. nidulans* to confirm PTS1 dependent import. Peroxins are proteins critical for peroxisome biogenesis (e.g. PexC) or protein targeting into peroxisomes (e.g. PexE). Furthermore peroxin mutant strains were compared to the wild type with respect to siderophore biosynthesis and growth rate during iron-replete and iron depleted conditions to show the role of peroxisomes in iron acquisition.

This is the first description of peroxisomal localization of siderophore biosynthetic steps beside known peroxisomal metabolic pathways as ß-oxidation and ergosterol biosynthesis.

PR1.41

The interplay of vacuolar and siderophore-mediated iron storage in the opportunistic fungal pathogen *A. fumigatus*

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Iron is an essential element for all eukaryotes but its excess is deleterious. Iron homeostasis results from tight regulation of iron acquisition and iron storage. *A. fumigatus* produces the extracellular siderophores triacetylfusarinine C (TAFC) and fusarinine C (FSC) for iron uptake and the intracellular siderophores ferricrocin (FC) and hydroxyferricrocin for iron distribution and storage. Siderophore biosynthesis is important for the adaptation to iron starvation and therefore crucial for virulence. Intracellular iron excess has been shown to increase the content of FC-chelated iron and the expression of AFUA_4g12530, termed CccA, which shows similarity to the vacuolar iron importer Ccc1 of *S. cerevisiae*. These data indicate a role of both the vacuole and FC in iron detoxification.

Green fluorescence protein-tagging confirmed localization of CccA in the vacuolar membrane. During high iron conditions genetic inactivation of CccA impaired growth, in particular in combination with derepressed iron uptake due to deficiency in the iron regulator SreA. In contrast, overproduction of CccA increased iron resistance. Inactivation of FC biosynthesis did not affect iron resistance. Lack of FC, CccA and in particular both, increased the cellular content of iron chelated by FSC/TAFC breakdown products. A delayed release of iron from FSC/TAFC degradation products might represent another iron detoxifying mechanism.

Our data indicate that vacuolar rather than FC-mediated iron storage is the major iron detoxifying mechanism of *A. fumigatus.*

PR1.42

Regulatation of the BUD3-BUD4 landmark complex by the NDR kinases DBF2 and COT1 during septum formation in *Neurospora crassa*

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Cytokinesis is essential for cell proliferation, yet the mechanisms for determining the cell division plane are only poorly understood. Our data indicate that the anillin BUD4 marks septum placement by organizing the RHO4-BUD3-BUD4 GTPase module and that this complex is controlled through two NDR kinase signaling cascades, the septation initiation network (SIN) and the morphogenesis network (MOR). By using a combination of live cell imaging, genetic and biochemical approaches, we show that COT1 and DBF2 localize to the constricting septum and are regulated by the two specific upstream germinal center (GC) kinases POD6 and NCU04096 that phosphorylate the respective NDR kinase at their C-terminal hydrobhobic motif. A third GC kinase, MST1, functions as generic regulator of both NDR kinases. *cot-1* and *dbf-2* mutants display opposite septation defects. Epistasis analysis of *sin*, *mor* and *bud* double mutants places the SIN upstream of the MOR, which in turn inhibits BUD function. We demonstrate that COT1, but not DBF2, binds to and phosphorylates BUD3 and BUD4. Mutational analysis of BUD3 identifies Ser798, located within an amphiphatic helix of BUD3 that seems to be phosphorylated by COT1. Localization of this amphiphatic helix at septa is only possible in its nonphosphorylated form. In summary, our data suggest a preliminary model, in which the MOR kinase COT1 phosphorylates BUD3 and BUD4 and that this phosphorylation inhibits cortical localization and function of the BUD complex.

PR1.43

ApsB, a component of microtubule-organizing centres resides in microbodies

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In *Aspergillus nidulans* septum-associated microtubule-organizing centres (sMTOCs) together with spindle pole bodies (SPBs) polymerize cytoplasmic microtubules. Previously, we identified a novel MTOC-associated protein, ApsB (*Schizosaccharomyces pombe* mto1), whose absence affected MT formation from sMTOCs more than from SPBs, suggesting that the two protein complexes are organized differently (Suelmann et al., 1998). sMTOCs share at least two further components, gamma-tubulin and GcpC (*S. pombe* Alp6) with SPBs, and ApsB interacts physically with gamma-tubulin. Surprisingly, ApsB contains a peroxisomal targeting sequence, PTS2, which could be replaced by a PTS1 motif at the C-terminus of ApsB (Zekert et al., 2010). Here, we show that fractions of ApsB and gamma-tubulin indeed reside in microbodies. Both proteins co-fractionated with microbodies in sucrose gradients. Protein protection assays revealed that ApsB and gamma-tubulin reside inside microbodies suggesting a novel role for these organelles in microtubule organization. As a further proof for such a function, we created a deletion mutant of *pexC*. PexC is an essential protein for peroxisomal biogenesis (Heiland & Erdmann, 2005). In agreement with our hypothesis, the *ΔpexC* strain partially phenocopied the *ΔapsB* mutation.

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PR1.44

Actin Precedes Myosin in Formation of Contractile Rings in *Aspergillus nidulans*

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Cytokinesis in filamentous fungi involves the progressive inward growth of the cell wall and plasma membrane, resulting in a series of functionally connected multinucleate compartments delimited by crosswalls termed septa. By screening chemically mutagenized strains of the model fungus *Aspergillus nidulans*, we have identified several septation-impaired isolates with mutations occurring in loci different from those of previously-identified *Aspergillus nidulans* "*sep*" mutations. Here we report that the lesion in one of these strains (RCH2) occurs in a gene that encodes a fungal homologue of mammalian myosin-II (MyoB; AN4706). Sequencing of the *MyoB* allele in the RCH2 strain identifies a point mutation predicted to result in a glycine-to-aspartate amino acid substitution at residue 843 in the myosin-II converter domain. This residue is conserved in all fungal, plant, and animal myosin-II sequences that we have examined. The mutation does not prevent localization of the MyoB protein to contractile rings, but it does block ring constriction. Wild type MyoB colocalizes with myosin light chain (MLC; AN6732), tropomyosin (TpmA; AN5686), and α-actinin (AcnA; AN7707) in contractile rings. Down-regulation of wild-type *MyoB* expression under control of the *A. nidulans AlcA* promoter blocks septation and localization of MLC to pre-septal rings, but not localization of actin or TpmA. Similarly, ring targeting of AcnA is blocked by the RCH2 mutation. Conversely, ring targeting of MyoB, AcnA, MLC, and TpmA are all blocked by disruption of filamentous actin using Latrunculin B. We propose, therefore, an "actin-first" model for the relationship between actin and myosin-II in formation of contractile rings.

PR1.45

Differential transcriptome and proteome analysis of the plant-pathogenic fungus *Verticillium longisporum*

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Verticillium longisporum is a soil-born host-specific fungal pathogen of *Brassicaceae*. It infects through the roots, colonizes the central cylinder inside the xylem vessels and spreads through the whole plant. The evolutionary origin of the cruciferous fungal pathogen, *V. longisporum* is still a mystery. It is very closely related to both *V. dahliae* and *V. albo-atrum* but possesses some typical characteristics such as long spores, almost double amount of nuclear DNA content and cruciferous host specificity. On the genomic level most of the genes of *V. longisporum* have two copies. In this project we focus on effectors of *V. longisporum* which modulate the interaction of the pathogen with its host *B. napus*. In order to identify such effectors, we started to compare biotrophic with saprophytic growth conditions. As we can't investigate the fungus within the plant yet, we extracted xylem-sap from oilseed rape and used it as growth substrate to mimic biotrophic conditions. We compared them with saprophytic growth media such as simulated xylem medium (SXM) and potato dextrose broth (PDB). All cultures were simultaneously used to analyze the transcriptome by RNA-Seq and the exoproteomes by MALDI-TOF and LC-MSMS. With this experimental setup we could identify several putative effectors which could play a role in the interaction of the fungus with the host. Currently, we are analyzing candidate genes with a knock-down strategy in *V. longisporum* and knock-out strategy in *V. dahliae*.

PR1.46

Localization Analysis of P-bodies and Stress Granules under Different Stress Conditions in the Filamentous Fungus *Aspergillus oryzae*

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Recently, it has become apparent that one aspect of posttranscriptional regulation of gene expression acts on the remodeling of translating mRNAs from polysomes into non-translating mRNPs (messenger ribonucleoproteins). P-bodies (processing bodies) and stress granules are two types of cytoplasmic mRNP granules that are widely observed in eukaryotes. Both of them are implicated in the posttranscriptional regulation of gene expression when cells are exposed to stresses. However, these structures in filamentous fungi including *Aspergillus oryzae* have not yet been elucidated in detail. In this study, the well-known components of P-bodies and stress granules were tagged with fluorescent proteins and analyzed in *A. oryzae* under various stress conditions. AoDcp2-EGFP and AoEdc3-EGFP-labeled P-bodies were observed in the cytoplasm under normal growth condition, and were further induced in response to stresses. AoPab1-EGFP and AoPub1-EGFP showed a dispersed distribution under normal growth condition, and the aggregation was induced to form stress granules under stresses. Most of the stress granules were colocalized or closely associated with P-bodies. Additionally, it has known that *A. oryzae* AoSO protein, a homolog of the *Neurospora crassa* SO, accumulates at septal pore in response to stresses. AoPab1 was also colocalized with the AoSO cytoplasmic foci induced by the high temperature stress, while the AoSO accumulation at the septal pore did not accompany with the AoPab1. The colocalization of the AoSO cytoplasmic foci with stress granules suggested that AoSO may have a novel role in the regulation of mRNP granules.

PR1.47

Contribution of MAK-1 and MAK-2 MAP kinases to cell wall integrity in *Neurospora crassa*.

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The Mpk1-like MAP kinases control the cell wall integrity that regulates cell wall remodeling during the growth and differentiation in fungi. *Neurospora crassa* has MAK-1 and MAK-2 MAP kinases which are an ortholog of Mpk1 and Fus3 in *S. cerevisiae*, respectively. MAK-2 regulates hyphal fusion and sexual development, whereas the function of MAK-1 is not fully understood. In this study, we examined the contribution of MAK-1 and MAK-2 to cell wall integrity in *N. crassa*. Both the *mak-1* and *mak-2* disruptants were more sensitive to micafungin, a beta-1,3-glucan synthase inhibitor, than the wild-type strain, whereas only *mak-1* disruptant displayed highly sensitivity to polyoxin, a chitin synthesis inhibitor. Western blot analysis revealed that the phosphorylation level of MAK-2 significantly increased by micafungin in wild-type strain. In contrast, the MAK-1 was constitutively phosphorylated from conidial germination to hyphal growth stage in wild type strain. Interestingly, this basal MAK-1 phosphorylation was almost abolished in the *mak-2* disruptant. When the mycelia of *mak-2* disruptant were treated with micafungin, the MAK-1 phosphorylation was significantly induced. These results suggest that both MAK-1 and MAK-2 participate in regulation of cell wall integrity, and there may be cross-talk between these MAP kinase pathways in *N. crassa.*

PR1.48

Genome wide insights into the targets and mechanism of function of LAE1 in *Trichoderma reesei*

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In *Aspergillus* spp., the *laeA* (loss of *aflR* expression) gene acts as global regulator of secondary metabolism. We found that the *T. reesei* orthologue LAE1 regulates cellulase and hemicellulase gene expression (Seiboth, Karimi e*t al.* unpublished data). In order to identify all targets of LAE1 in *T. reesei*, we analysed the transcriptome of *lae1* deletion (*Dlae1*) and LAE1 overexpressing (OE) strains during growth on lactose. 1186 (13 % of the total 9143) genes of *T. reesei* were affected more than 2-fold in the *Dlae1* strain, and 701 in the OE strain, and almost all of the latter (696) were also significantly expressed in the former strain. Amino acid permeases, ankyrins, and G-protein coupled receptors were most strongly downregulated in *Dlae1* and upregulated in OE. Expression of known (PKS, NRPS) or predicted (monooxygenases, dioxgenases, multicopper oxidases) enzymes involved in secondary metabolite production was also affected, with roughly equal numbers of genes being up- and downregulated. Approximately 15 % of the genes presumably regulated by LAE1 occurred in 40 discrete clusters in the *T. reesei* genome. ChIP-seq analyses suggest that LAE1 does not directly alter the balance of an active (H3K4me2) and silencing (H3K9me3) histone modification under our conditions. Conidiation was strongly reduced in the delta-*lae1* strain, both in light and in darkness, and about one third of conidiation genes were affected in the *Dlae1* strain, indicating a major effect of LAE1 on *T. reesei* sporulation. Our data expand the current knowledge of LAE1 function and point to a major involvement in conidiation in *T. reesei*.

PR1.49

LAE1 regulates conidiation in *Trichoderma atroviride*

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Trichoderma atroviride (teleomorph *Hypocrea atroviridis*) exhibits a synchronized manner of sporulation in response to light stimulus, and thus serves as a model organism for photomorphogenetic investigations. However, conidiation of *Trichoderma atroviride* strongly depends on carbon source. Here we show the involvement of the LAE1 protein in light sensing by *T. atroviride* and regulation of its development. Phylogenetic analysis shows that LAE1 from *T. atroviride* is orthologous to LAE1 from *T. reesei* (teleomorph *Hypocrea jecorina*) and they both are orthologous to LaeA from *Aspergillus nidulans* (teleomorph *Emericella nidulans*). In the later fungus this protein is the master regulator of secondary metabolite production. A loss of function of *lae1* in *T. atroviride* dramatically reduces sporulation in general and weakens the induction of conidia formation by light. We compare photostimulation of conidiation of *lae1* mutants of *T. atroviride* P1 (*lae1* knockout and *lae1*OE respectively) and the corresponding *lae1* mutants of *T. reesei* by using a broad set of carbon sources in an optimized BIOLOG Phenotype MicroArray. We also show that the two blue light receptor proteins BLR-1 and BLR-2 interact with LAE1 in the developmental regulation of *T. atroviride*. Last not least, the mechanical injury of mycelia, which usually triggers the conidiation in *T. atroviride*, was not functional in *lae1* knockout mutant as compared to parent and *lae1*OE strains. These data suggest that developmental regulation is a main target of LAE1 function in *T. atroviride*.

PR1.50

Cytological observation of mitotic chromosomes in *Colletotrichum* spp. and its implications for chromosome analysis, genome project and phylogenetics

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In this study, we show that cytological approach is useful to analyze chromosome structure, karyotype, and phylogeny of *Colletotrichum* spp. Seven species, *C. graminicola* (*C-gra*), *C. higginsianum* (*C-hig*), *C. orbiculare* (*C-orb*), *C. gloeosporioides*, *C. lindemuthianum* (*C-lin*), *C. truncatum*, and *C. trifolii* (*C-tri*), were examined here. For visualizing chromosomes, mitotic metaphase specimens were prepared by germ tube burst method, stained with DAPI and PI, and observed by fluorescence microscopy. Summarized results are as follows. (1) Using *C-gra* and *C-hig*, cytological karyotypes were shown to be consistent with optical maps in terms of chromosome number (CN) and relative chromosome size. Even mini-chromosomes of 400-500 kb in optical maps were clearly observed with our method. With the merits of cost and time, cytological karyotyping should be useful to know the outline of genome at the onset of genome project. (2) In *C-orb*, a novel feature of chromosomes was discovered: most chromosomes are distinctly partitioned into highly AT-rich regions that constitute centromeric, constitutive heterochromatin and the remaining highly GC-rich regions. Also chromosome rearrangements were easily detectable between the strains based on morphological features. Thus, *C-orb* is promising as a model for fungal cytogenetics. (3) Comparison of karyotypes and cytological features of interphase nuclei among seven species indicated that *C-orb* and *C-tri* are very close to each other with similar cytological properties unique to them, but are distantly related to other species including *C-lin*. This finding suggests that *C-orb* and *C-tri* diverged recently. Core CN excluding mini-chromosomes were 7 for *C-lin* and 10 for the other species.

PR1.51

Regulation of the expression of genes encoding glycoside hydrolases in *Penicillium funiculosum*

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Penicillium funiculosum is a filamentous fungus that has the capability to secrete a mixture of enzymes (Rovabio™) that is used as animal feed additive for the enhanced hydrolysis of plant polymers. The aim of this work is to study the networks regulating the production and secretion of the hydrolytic enzymes that constitute the cocktail from *P. funiculosum*. The genome of *P. funiculosum* was sequenced and, following computer-based annotation, a manual annotation has been initiated with a focus on genes likely to encode glycoside hydrolases, regulators and proteins involved in the secretion pathway. Furthermore, we have constructed a Δ*pyrG* strain of *P. funiculosum* to afford gene deletion studies as part of a programme to assess the functionality of target genes. We studied the expression of selected genes encoding glycoside hydrolases that are known to be present in the Rovabio™ cocktail. As a prelude to a genome-wide transcriptional analysis, we studied the transcription of genes encoding a cellobiohydrolase, an arabinofuranosidase and a xylanase using semi-quantitative RT-PCR to determine the level of transcripts from cells grown in presence of two different carbon sources, glucose and wheat straw. For those genes, transcription was repressed in glucose and induced in wheat straw medium. With the tools now assembled, we are in a strong position to investigate the regulation of gene expression in *P. funiculosum*.

PR1.52

Subcellular loclisation of AREA and AREB under different carbon and nitrogen regimes.

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Nitrogen metabolite repression modulates the expression of target genes participating in utilization of alternative nitrogen sources, resulting in transcription only when glutamine or ammonium levels are limiting. In Aspergillus nidulans this regulatory mechanism depends on GATA transcription factors AREA and AREB. Both these factors function as a repressor of arginine catabolism genes under nitrogen repressing conditions.The activities of AREA and AREB are differentially regulated by the carbon regime: AREA being necessary for the ammonium repression these genes under carbon repressing conditions, while AREB is primarily involved under carbon-limiting conditions. To invastigate how a subcellural localistion of these two regulators depends on carbon and nitrogen regimes, A. nidulans strains expressing AREA and AREB fusions with fluorescent proteins were made and localisation of these two proteins detected under different nitrogen and carbon conditions. Bimolecular Fluorescent Complementation (BiFC) system was also used to determine interactions of AREA and AREB. We also transformed A. nidulans areB deletion mutant with areB gene from plant pathogen Fusarium fujikuroi to check if both proteins are functional homologues.

PR1.53

The fungal plasma membrane: a central player in the toxicity of the *Penicillium chrysogenum* antifungal protein PAF

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The cationic antifungal protein PAF inhibits the growth of sensitive filamentous fungi, e.g. *Aspergillus nidulans*, *Aspergillus fumigatus*, *Neursopora crassa*. The plasma membrane plays a crucial role in binding PAF, regulating ion channels in response to PAF, transmitting signals into the cell and finally also in PAF uptake. Thus the plasma membrane might determine at least in part PAF resistance or sensitivity. A microarray-based gene expression analysis in *A. fumigatus* revealed the deregulation of genes in response to PAF which are directly involved in fatty acid and lipid synthesis, membrane composition and cell signalling. In accordance, the following pathways were identified to be significantly deregulated by PAF treatment (KEGG GSEA, $p<0.05$): glycerophospholipid metabolism (afm00641), inositol phosphate metabolism (afm00562) and phosphatidylinositol signalling (afm04070). A change in the expression of genes involved in the synthesis of the plasma membrane may affect its composition and fluidity and finally its accessibility for PAF pointing towards the attempt of the fungus to survive the antifungal attack. We studied in more detail the interaction of PAF with the plasma membrane and found that PAF binds exclusively to phosphoinositolphosphate (PIP), bis- (PIP_2) and triphosphates (PIP_3) and phosphatidic acid (PA), but not to phospholipids. These properties were further investigated by solution NMR ^{15}N-chemical shift titration (CST) and saturation transfer difference (STD). We could prove by CST that PAF binds to PIP_3 with moderate affinity (K=3.600/M). STD experiments revealed that PAF interacts by its aromatic parts with so far unidentified membrane components (putatively high molecular weight proteins).

PR1.54

New insights into the regulation of candidate effector proteins of the fungal wheat pathogen *Mycosphaerella graminicola*

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The ascomycete *Mycosphaerella graminicola* is an economically important wheat pathogen causing septoria tritici blotch (STB), which is one of the most devastating wheat diseases worldwide. To establish successful infection, this fungus secretes a wide array of small proteins to evade or escape host resistance mechanisms during colonization. Identification and functional characterization of genes involved in effector secretion render new insights in the genetic control of pathogenicity and may lead to the identification of novel effector proteins. Here, we show that *MgWor1* is an important regulatory gene in *M. graminicola* that plays a crucial role in the expression of specific small-secreted proteins (SSPs), either directly or indirectly through links with signal transduction pathways. For instance, we found that expression of several SSPs is controlled by *MgTpk2*, the catalytic subunit of protein kinase A (PKA), or the G protein *MgGpb1* that regulates the cyclic AMP pathway and is required for pathogenicity of *M. graminicola*. Functional analyses of these candidate effectors are ongoing and will be discussed.

PR1.55

Functional characterization of three HMG-CoA reductase genes in the beta-carotene producing *Mucor circinelloides*

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In fungi, carotenoid biosynthesis branches from the general acetate/mevalonate pathway. 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is a key enzyme of this pathway catalysing a rate-limiting step, the conversion of HMG-CoA to mevalonate. The *Mucor circinelloides* genome contains three HMG-CoA reductase genes (named in this study as *hmgR1*, *hmgR2*, *hmgR3*). All of them were cloned based on sequence data available in the *M. circinelloides* genome database (http://genome.jgi-psf.org/Mucci2/Mucci2.home.html) and used in gene expression studies to investigate their role in the carotenogenesis and other physiological and cell biological processes.

Using autonomously replicating plasmids, a set of transformants were constructed, in which the copy number of each of these genes were elevated. Gene-dose effect enhanced the carotenoid production and decreased the sensitivity to statins (inhibitors of the HMG-CoA reductase enzyme) of all types of transformants, but in different extent. Relative transcription levels of the three genes during the life cycle of the fungus and under different cultivation conditions (such as aerobic/anaerobic growth or different carbon sources) were analysed by quantitative real-time PCR. In these studies, *hmgR1* showed a constitutively low, while *hmgR2* showed a constitutively high transcription level. Transcription of *hmgR3* increased significantly under anaerobic growth conditions. Moreover, growth rate of transformants, in which *hmgR3* was silenced, showed a reduced growing rate. Our results suggest that *hmgR2* may play an important role in the general metabolism, while *hmgR3* may have a role in the sensing of the oxygen concentration and the mycelial development.
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PR1.56

Functional analysis of Hsp70 family protein SsaA in *Aspergillus oryzae*

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One of the possible mechanisms of azole resistance is the up-regulation of genes encoding drug efflux pumps, mainly belonging to ABC transporters. *Aspergillus oryzae* exhibits azole drug resistance through overexpression of ABC transporter genes such as *atrA*, *atrF*, and *atrG*. Expression of these transporter genes is regulated by a Zn(II)Cys6 transcription factor, AtrR. Although it was suggested that AtrR shows different responses dependent on azole drug species, the detailed mechanism underlying regulation of AtrR in expression of the ABC transporter genes has not been elucidated. Since in *Saccharomyces cerevisiae* the AtrR counterpart Pdr1/Pdr3 are associated with Mediator protein complex, we examined whether or not there are Mediator-like proteins or co-activators interacted with AtrR in *A. oryzae*. To identify such proteins, a tandem affinity purification (TAP) moiety was fused to AtrR, with which candidate proteins prepared from the fungal mycelium were co-immunoprecipitated. The tandem mass spectrometry analysis showed that one of proteins enriched in the co-immunoprecipitated fraction was Hsp70 family protein, an ortholog of yeast Ssa1 (SsaA). In *S. cerevisiae* Pdr1 is positively regulated by Hsp70 protein Ssz1 and Pdr3 is negatively regulated by Ssa1/2. We constructed an overexpression strain of *ssaA* in *A. oryzae* and examined the involvement of the gene in the drug resistance. Overexpression of *ssaA* resulted in a slight decrease in azole drug resistance. In addition, this strain also showed a decrease in the expression of ABC transporter genes (*atrA*, *atrF*, and *atrG)*. These results suggested that AtrR is negatively regulated by SsaA in *A. oryzae*.

PR1.57

Isolation and characterisation of *FcStuA* in *Fusarium culmorum*, causal agent of foot crown rot

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F. culmorum is the causal agent of crown foot rot (CFR) as well as of Fusarium head blight (FBH) on wheat. Here we report on the characterisation of *FcstuA* gene, a APSES protein, in the *F. Culmorum* genome. The *FcstuA* gene was deleted by homologous recombination in two wild-type strains which are able or unable to produce deoxynivalenol *in vitro*, respectively. Both deletion mutants showed complete loss of pathogenicity in a CFR pathosystem on durum wheat being also unable to efficiently colonise different plant tissues (apple, potato and tomato). Fungicidal assays showed that the mutants are equally sensitive to three classes of fungicides as the wild type. Other phenotypes of the mutants include impaired growth on solid substrates, loss of hydrophobicity of the mycelium, higher susceptibility towards oxidative stress, lack of monophialides and altered pigmentation. Toxin production was decreased but not completely inhibited as in *F. Graminearum*. It is argued that toxin production is not essential for determining the loss of pathogenicity by *FcstuA* mutants in CFR. Data obtained by environmental Scanning Electron Microscope analysis suggests that the hyphae of the mutant are unable to penetrate the germinating grain.

PR1.58

To kill a fungus- antifungal activity of a plant defensin

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Plant defensins are small, cysteine-rich proteins involved in the innate immune system of plants. The plant defensin NaD1, from the ornamental tobacco Nicotiana alata is highly expressed in flowers and has a role in fungal protection. Its potent antifungal activity has been used to protect transgenic cotton from fungal infections in the field. The antifungal activity of NaD1 involves a multi-step mode of action whereby NaD1 interacts specifically with the cell wall, permeabilises the plasma membrane and ultimately enters the cytoplasm. The kinetics of cell wall binding and membrane permeabilisation suggest that a cell wall receptor may be required. In an attempt to identify this receptor as well as any other genes that may be required for the antifungal activity of NaD1, we are screening a Fusarium oxysporum mutant library created by random TDNA mutagenesis. Mutants that demonstrate enhanced resistance to NaD1 will be characterised to identify the gene(s) responsible for the resistant phenotype. Mutants will also be examined for changes in the cell wall composition or proteome in order to better understand the mechanism of resistance.

PR1.59

Rho GTPases: Insights From The *Schizophyllum commune* Genome

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The sequenced genome of the mushroom *Schizophyllum commune* provides a powerful tool to inventory the components of different signalling pathways in filamentous basidiomycetes. Six Rho GTPases could be identified in the *S. commune* genome, namely Rho1, Rho2, Rho3, Rho4, Cdc42, and Rac1. *S. commune* Rho3, Cdc42 and Rac1 have been previously cloned (cDNA sequences with accession numbers AAF61254, AY954038, AAQ88447 in GenBank), while *S. commune* Rho1, Rho2 and Rho4 were annotated as hypothetical Ras-related small GTPases of the Rho subfamily during the genome project. Only one copy of each gene is detected in the genome, as has been suggested previously for *Rho3*, *Cdc42* and *Rac1* by Southern analysis. In addition the *S. commune* genome contains 11 and 12 genes encoding RhoGAP and RhoGEF proteins, respectively, but only one gene for RhoGDI. Ectopic expression of constitutively active *cdc42* in *S. commune* changes the hyphal regions at septa into multinucleate, swollen compartments with disturbed branching. These alterations suggest that Cdc42 is regulating branch formation and growth in *S. commune*. The tips of hyphae at the edge of *S. commune* colonies that are expressing constitutively active *cdc42* have a normal morphology, suggesting that Rac1 rather than Cdc42 regulates the polarized growth of leading hyphae. This hypothesis is now under cell biological investigation in living hyphae. No effect of expression of constitutively active or inactive *Rho3* on hyphal growth was observed but in Northern hybridization with *Rho3* cDNA a signal was obtained in young haploid hyphae and a slight increase during their mating interaction.

PR1.60

Filamentous septin in the corn smut fungus *Ustilago maydis* is specifically recognised by Gap7, a novel Cdc42 specific GTPase-activating protein

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Cell separation in fungi requires the formation of plate-like cross-walls called septa. A key determinant during septum formation is the assembly of septins into a ring-like structure, for which septins need GTP-binding. In the plant pathogenic fungus *Ustilago maydis* septins form collar-like, ring-like and filamentous structures. In the genome of *U. maydis* four septins have been identified: Sep1-4. Interestingly, only Sep4 (the Cdc10 ortholog of S. cerevisiae) is able to assemble in long filaments.

Recently, we have started to investigate a class of proteins, the GTPase-activating proteins called GAPs towards their specificity on the Rho/Rac-like GTPases in *U. maydis*. We found a novel type of GAPs with the ability to bind specifically to filamentous septin Sep4. Overexpression of the GAP-domain of this Gap7 protein is able to switch off active Cdc42 leading to a cell separation defect known for *cdc42* mutants in *U. maydis*. Interestingly, beside the GAP-domain the amino acid sequence of Gap7 contained no additional domain after searching the protein databases.

To narrow down the region which enables septin binding we have started to generate deletion mutants fused to GFP for colocalisation studies with cdc10-RFP. These mutants were also tested in GST-pulldown-assays with GDP- or GTP-bound Sep4. Our data showed that the Gap7-GAP domain was also able to interact with Cdc10. Currently we are investigating if Gap7 is a real GAP for Cdc10 and what is the function of filamentous septin in *U. maydis*.

PR1.61

Insights into the structural determinants for specificity and transport by UreA, the specific urea transporter of *Aspergillus nidulans*

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UreA, is a high-affinity urea/H+ symporter which seems to be the sole transport system specific for urea in*Aspergillus nidulans*. Homologous proteins have been characterized in *Saccharomyces cerevisiae*, *Paxillus involutus*, *Candida albicans,* in the model plant *Arabidopsis thaliana* and more recently in *Oryza sativa* (rice).Paralogues are present in the genomic sequences of all *Aspergilli*. The protein is predicted to consist of 15 transmembrane helical domains (TMSs), with an extracellular N-terminus and an intracellular C-terminus.

Little is known about the structure-function relationship of these membrane proteins. In order to address this subject, we designed a mutational strategy based on 3D homology modelling of UreA and the identification of conserved residues in all known fungal urea transporters. The functionality of the mutant proteins was assayed by growth tests on urea and resistance to its toxic analogue, thiourea. All mutations were introduced on an UreA::GFP fusion construct, which allowed us to follow the sub-cellular localization of mutant fusion proteins. This strategy allowed us to identify a number of key residues involved in the recognition and/or translocation of urea across the membrane. These mutations localize in helixes number 3, 7 or 11 which, according to homology modelling, are predicted to be part of the substrate binding domain. A chemical mutagenesis approach has been also undertaken which allowed for the identification of key residues for the functionality of the protein.

Our work constitutes the first mutational analysis in this family of transporters, providing insights into urea transporters functionality.

PR1.62

The Role of Ornithine Supply in Siderophore Biosynthesis in *Aspergillus fumigatus*

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Background: Iron is an essential nutrient required for a wide range of cellular processes. However, excessive iron accumulation is toxic. Therefore, microorganisms evolved fine-tuned iron uptake and storage mechanisms, such as the siderophore system. The opportunistic fungal pathogen *Aspergillus fumigatus* produces siderophores (low-molecular mass iron-specific chelators) to acquire, store and distribute iron. Past studies indicated coordination of siderophore biosynthesis with supply of its precursor ornithine.

Methods: The role of mitochondrial ornithine production in siderophore biosynthesis of *A. fumigatus* was characterized by analysis of the phenotypic consequences of genetic inactivation of the putative mitochondrial ornithine exporter, AmcA (Afu_8g02760).

Results: Consistent with a role in mitochondrial ornithine export, inactivation of AmcA resulted in a decrease in the cellular ornithine content as well as a decrease in extra- and intracellular siderophore production. In the presence of the iron chelator bathophenanthroline disulfonate, which inhibits siderophore-independent iron uptake, AmcA-deficiency decreased conidiation, indicating increased iron starvation. In contrast to siderophore production, AmcA-deficiency didn't affect the cellular content in polyamines, which are also derived from ornithine via the ornithine decarboxylase. Nevertheless, AmcA-deficiency increased the susceptibility of *A. fumigatus* to eflornithine, an inhibitor of the ornithine decarboxylase, most likely due to the decreased ornithine pool.
Conclusion: Siderophore biosynthesis is mainly fueled by mitochondrial production of ornithine, rather than by conversion of arginine to ornithine in the cytoplasm. There exists coordination between siderophore biosynthesis and its precursor supply. This study also indicates a prioritization of ornithine flux into synthesis of polyamines compared to siderophores, emphasizing the essentiality of polyamines.

PR1.63

Disruption of the catalytic subunit of calcineurin (*cnaA*) in the grass symbiont *Epichloë festucae* reduces host colonization and induces formation of intrahyphal hyphae both in culture and *in planta*

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Calcineurin is a Ca^{2+}/calmodulin-dependent protein phosphatase (PP2B) comprised of a catalytic subunit (CnaA) and a regulatory subunit (CnaB). Calcineurin signalling, which occurs via the calcineurin-responsive transcription factor CRZ1, is important for regulation of ion stress, cell wall integrity, hyphal growth and a number of other developmental processes including those associated with fungal-plant interactions. The aim of this project is to test whether calcineurin has a signalling role in establishment of the mutualistic symbiotic interaction between *Epichloë festucae* and *Lolium perenne*. Analysis of the genome sequences of *E. festucae* strains Fl1 and E2368 identified one *cnaA* copy in the former and two in the latter. Deletion of *cnaA* in Fl1 resulted in severe defects in culture morphology and growth. TEM revealed a remarkable developmental defect; hyphae of the mutant formed intra-hyphal hyphae. These growth defects were partially remediated by plating mycelia at high density or by growing in the presence of $MgCl_2$. The two copies of *cnaA* in E2368 were shown to be functionally redundant. Both *cnaA1* from Fl1 and *cnaA2* from E2368 were able to complement the D*cnaA* mutant phenotype. Inoculation of *L. perenne* seedlings with the D*cnaA* mutant resulted in a strong hypersensitive response (HR). By contrast, remediated cultures failed to induce an HR but were still defective in host colonization. In the few plants that were colonized hyphal biomass was reduced and intra-hyphal hyphae were observed in the intercellular spaces of the leaves. This work demonstrates that calcineurin signalling is crucial for symbiotic establishment and for normal hyphal tip growth and development.

PR1.64

***Candida albicans* biofilms and their risk in nosocomial infections**

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The hospital can be considered as an ecosystem where the patient is in contact with the microbial world and faces the risk of contracting an infection that is termed the nosocomial. Some yeasts parts of this universe, like *Candida albicans*, are opportunist pathogens. Usage of catheters provides ample opportunity for *C. albicans* biofilms to set up a nidus for disease that is not easily amenable to conventional antifungal therapy.

Nosocomial infections caused by bacteria associated with medical implants, especially catheters, have been the subject of intense researches for a long time in Algeria, but the infections of fungal origin, particularly those caused by *C. albicans* have not been clarified yet. For this framework, our study was done at Maghnia hospital (Algeria), where 51 strains (16.94% of all taken samples) of *C. albicans* were isolated. They were divided into various hospital services with variable rates; the most concerned is the ICU followed by the gynecology department, while that of general surgery came third.

Furthermore, testing the antifungal amphotericin B "AmB" showed clearly that the *C. albicans* sessile cells (in biofilms) are much more resistant than their planktonic counterparts (suspended cells) that the resistance increases during the different phases of biofilm formation until it reaches its threshold at the maturation phase (48 hrs).

PR1.65

Assessment of alterations types of catheters by *Candida spp*. in ICU. First study in Algeria

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Nosocomial candidiasis remain a potential risk in the Intensive Care Units (ICU) where *Candida albicans* is the most responsible; however, non-albicans *Candida* species, especially *C. glabrata*, are involved. These infections are often associated with biofilms that contaminate the medical implants such as catheters; thus, therapeutic failures are due to their increased resistance to antifungal agents.

The diagnosis of catheter-related candidiasis is difficult; however, the differentiation between catheter infection (or other medical implant) and a simple contamination is essential to establish an antifungal treatment.

Our study was conducted between February 2011 and Jun 2011 in the ICU of Sidi Bel Abbes University Hospital Center (Algeria). We evaluated the various types of catheters alterations (contaminations, colonisations and infections) and their corresponding rates, as well as the responsible yeast species. In addition, we have taken images (photography) from a patient (tongue and the breathing tube) showing the spread of biofilms of *C. albicans*. In parallel, we have enhanced our study by conducting a prevalence survey by including several sampling sites.

PR1.66

Dynamics of horizontal chromosome transfer in *Fusarium oxysporum*

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Fusarium oxysporum (*Fo*) is known as a diverse and widely dispersed pathogenic species complex showing a broad host range, including many economically important crops. Comparative genomics revealed lineage-specific (LS) genomic regions in *Fusarium oxysporum f. sp. lycopersici* (*Fol*) that include four entire chromosomes and account for more than 25% of the genome. At least two LS chromosomes can be transferred horizontally to non-pathogenic *Fo* strains, resulting in pathogenicity towards tomato in the recipient.

To unravel the mechanics of horizontal chromosome transfer we will use the live-cell fluorescence system developed by Ruiz-Roldan et al. to first observe nuclear dynamics during hyphal fusion events between chromosome donor and acceptor strains. Subsequently, the split-GFP technique will be established to (i) examine temporal and spatial distribution of hyphal fusion between different donor and acceptor strains and (ii) determine whether LS chromosomes are transferred *via* nuclear fusion or exit the donor and enter the acceptor nucleus.

PR1.67

Redirection of pigment biosynthesis to isocoumarins in *Fusarium*

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Colonies of *Fusarium* species often appear red due to production of pigments, such as bikaverin or aurofusarin. The entry compounds in the aurofusarin and bikaverin biosynthetic pathways are YWA1 and pre-bikaverin, respectively, in a process catalyzed by two multidomain polyketide synthases (PKSs), which both have a claisen-type cyclase domain (CLC) in their N terminal. Disruption of the CLC domains has previously been shown to result in formation of the lactones citreoisocoumarin and SMA93 instead of YWA1 and pre-bikaverin, respectively.

In the present study we have developed a medium with low nitrogen supply on which the aurofusarin or bikaverin pathways were partially redirected resulting in production of citreoisocoumarin and SMA93, respectively. This is first time that SMA93 is identified in a fungus and we suggest that it is renamed bikisocoumarin, as it is derived from the bikaverin pathway. The redirection of the aurofusarin and bikaverin biosynthetic pathways was reverted by adding nitrate or ammonium to the medium, suggesting that nitrogen starvation induces isocoumarin production. The suppressive influence of nitrate was investigated using *Fusarium graminearum* where even small amount of nitrate (0.1 g/L $NaNO_3$) more than halved the production of citreoisocoumarin and was undetectable at 3 g/L $NaNO_3$.

PR1.68

Effect of conditional expression of CreA and HECT ubiquitin ligase HulA on glucose repression in Aspergillus oryzae

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Aspergillus oryzae has an ability to produce copious amounts of amylolytic enzymes, production of which is repressed in the presence of glucose. Glucose repression in filamentous fungi is mediated by the transcriptional repressor CreA. In Aspergillus nidulans, it has been proposed that ubiquitination and deubiquitination of CreA play a key role in regulating glucose repression, and that HECT ubiquitin ligase HulA is involved in ubiquitination of CreA. Since yeast ortholog (Rsp5) of HulA is an essential factor for cell viability, we speculated that HulA is also essential for cell viability in filamentous fungi. In addition, it has been known that deletion of creA has a detrimental effect on growth, although CreA is not essential for cell viability. Thus, we generated the conditional expression strains for CreA and HulA in A. oryzae to investigate the mechanism of glucose repression. The conditional expression strains were generated by using the promoter of nmtA, expression of which is repressed considerably in the presence of thiamine. The resultant conditional HulA expression strain was defective in conidial formation in thiamine-containing medium, suggesting that HulA is essential for conidiation or cell viability. On the other hand, the growth defect of CreA conditional expression strain in thiamine-containing medium was leaky as compared with the creA null mutant. Northern blot analysis of α-amylase gene showed that glucose repression was relieved in the CreA conditional expression strain. These results suggested that suppression of CreA expression level is highly effective in relieving the glucose repression without growth defect.

PR1.69

Specificity determinants of GTPase recognition by RhoGEFs in *Ustilago maydis*

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Small GTPases of the Rho family act as molecular switches and are involved in the regulation of many important cellular processes. They are activated by specific guanine nucleotide exchange factors (RhoGEFs). In their active GTP bound state RhoGTPases interact with downstream effectors and trigger cellular events. The number of both RhoGEFs and effectors exceeds the number of GTPases, which raises the question how signalling specificity is achieved. In recent years the importance of RhoGEF specificity became more and more evident, as these upstream activators are often connected to their downstream effectors by scaffolding proteins. We analysed all *U.maydis* Cdc42-specific RhoGEFs (Don1, Its1 and Hot1) for their role in Cdc42 signalling both in vivo and in vitro. Interestingly, the GTPase recognition mechanism differs between Hot1 and the other two RhoGEFs. While amino acid at position 56 of Cdc42 is critical for GEF recognition of Don1 and Its1, Hot1 uses a different amino acid to discriminate between Cdc42 and Rac1. We identified additional amino acids which are important for GTPase recognition by Hot1. In future we will try to find out whether orthologs of Hot1 in other organisms use a similar mechanism to discriminate between GTPases.

PR1.70

***Cryptococcus neoformans SEC7-1* is involve in high temperature growth**

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The basidiomycete yeast *Cryptococcus neoformans* is an opportunist pathogen that causes life threatening disease on immune-compromised patients. The treatment of choice for this mycosis is based on azoles; but clinical strains resistant to the treatment have been reported. Therefore, it is desirable that new treatment alternatives are developed and to do so, is necessary to increase the knowledge on this fungus biology. To explore this alternative, a random insertion mutagenesis library was created aiming to uncover genes involved in high temperature growth (37°C) which is an important virulence factor in *C. neoformans*. Among several mutants unable to grow at 37°C we have found *Sec7*. This gene encodes a guanine nucleotide exchange factor protein (GEF) that activates ADP ribosylation factor (ARF) which is a key regulator of vesicular transport in eukaryotic cells. *C. neoforman* genome has two genes that code proteins with a Sec7 domain and both are called Sec7. However, they lie on different chromosomes, 1 and 5 (*Sec7-1* and *Sec7-2*, respectively). Previously, in *S. cerevisiae* two SEC7 coding genes were identified but the deletion of only one of them actually impaired growth at the restrictive temperature. The second gene was able to correct this defect only when over expressed. Our results suggest that deletion of *C. neoformans Sec7-1* gene is sufficient to impair the growth at 37°C.

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PR1.71

Germination of conidia of *Aspergillus niger* is accompanied by major changes in RNA profiles

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The transcriptome of conidia of *Aspergillus niger* was analysed during the first 8 hours of germination. Dormant conidia started to grow isotropically 2 hours after inoculation in liquid medium. Isotropic growth changed to polarized growth after 6 hours, which coincided with one round of mitosis. Dormant conidia contained transcripts from 4626 genes. The number of genes with transcripts decreased to 3557 after 2 hours of germination, after which an increase was observed with 4780 expressed genes 8 h after inoculation. Dormant conidia had the most unique RNA composition. The correlation coefficient between the RNA profiles of t = 0 h and t = 8 h was 0.46. They were between 0.76-0.93 when profiles of 2, 4 and 6 h were compared with that of 8 h. Dormant conidia were characterized by high levels of transcripts of genes involved in the formation of protecting components such as trehalose, mannitol, protective proteins (*e.g.* heat shock proteins and catalase). Transcripts belonging to the Functional Gene Categories (FunCat) protein synthesis, cell cycle and DNA processing and respiration were over-represented in the up-regulated genes at t = 2, whereas metabolism and cell cycle and DNA processing were over-represented in the up-regulated genes at t = 4 h. At t = 6 h and t = 8 h No functional gene classes were over- or under-represented in the differentially expressed genes. Taken together, it is concluded that the transcriptome of conidia changes dramatically during the first two hours and that initiation of protein synthesis and respiration are important during early stages of germination.

PR1.72

Identification and Characterization of a *Trichoderma reesei* Calcofluor-Sensitive Mutant

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Genencor/DuPont

As a filamentous fungus capable of secreting large amounts of extracellular proteins, *Trichoderma reesei* is used for large-scale production of native as well as heterologous proteins. As such, it is of interest to explore changes in the *T. reesei* genome that have the potential to affect its secretion capability. This study presents the characterization of a calcofluor-sensitive mutant obtained by mutagenesis. We identified the mutation responsible for the phenotype using complementation cloning, CGH analysis and genome sequencing. The mutation resides in a gene encoding a protein with a putative function in ER-Golgi trafficking. The findings of this study help better understand the phenotypic consequences of mutations in genes encoding components of the secretion pathway in *T. reesei*.

PR1.73

Genetics of horizontal chromosome transfer in the plant pathogenic fungus *Fusarium oxysporum.*

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The *Fusarium oxysporum* species complex is comprised of many host specific asexual lineages. Interestingly, different lineages that infect a single host, reside in different phylogenetic clades. This distribution of host specificity has been attributed to acquired pathogenicity. Lineages sharing a host share chromosomes and genomic regions carrying effector genes. Transfer of these regions could explain the distribution of host specific lineages within the species complex.

Horizontal transfer of complete chromosomes has been demonstrated under laboratory conditions, and resulted in gain of pathogenicity by the non-pathogenic recipient. Using a genetics approach we aim to uncover the underlying cellular processes, which have so far remained elusive. We are currently creating knockout strains in genes with key functions in processes thought be required for horizontal chromosome transfer.
Furthermore, we will investigate what properties make a chromosome amenable for transfer. A screening method based on random insertion of a marker in the donor strain, will allows us to determine which chromosomes can be transferred. Analysis of these chromosomes should give us better insight into which properties they share.
Combining knowledge of both the properties required for chromosome transfer and the cellular processes involved will help to gain a better understanding of this process and its importance in the evolution of the *F. oxysporum* species complex.

PR1.74

The RNA-binding protein Khd4 - a posttranscriptional regulator for cell morphology and pathogenicity in *Ustilago maydis*

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In the plant-pathogen *Ustilago maydis* evidence is accumulating that posttranscriptional processes play a major role in regulating cell morphology and pathogenicity. Key factors of the posttranscriptional machinery are RNA-binding proteins, which recognize specific motifs within target transcripts to regulate for example translation, localization, or mRNA-stability. We are working with the RNA-binding protein Khd4 that contains at least five K homology domains. Deletion of *khd4* leads to severe consequences: disturbed cell shape, abnormal cell wall composition, cytokinesis defect, and strongly reduced pathogenicity. Interestingly, the KH domains 3 and 4, which recognize the motif AUACCC, are required for Khd4 function since mutations in the conserved motif G-X-X-G lead to the *khd4* deletion phenotype. The motif AUACCC is necessary and sufficient for binding and is most likely a regulatory element since it accumulates in untranslated regions. An independent mRNA expression profiling approach revealed that the binding motif is significantly enriched in transcripts showing altered expression levels in *khd4*Δ strains. Moreover, the vast majority of potential Khd4 target mRNAs exhibit increased amounts in deletion mutants. These findings suggest that Khd4 might function in mRNA-stability processes and is important for the posttranscriptional regulation of cell morphology and pathogenicity in *U. maydis*. In preliminary studies we analyzed truncated versions of Khd4 to learn more about putative domains in this large protein. In addition, we identified one of the regulated candidates that show the same deletion phenotype like the *khd4Δ* strain.

PR1.75

Analysis of G-protein and MAPK-components of the pheromone signal transduction cascade in *Ashbya gossypii*

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MAP-kinase cascades are highly conserved among eukaryotes and harbour key virulence factors in human- and plant pathogens. In sexually reproducing fungi the pheromone-signal transduction MAPK-cascade regulates mating and sporulation. The genome of the filamentous fungus *Ashbya gossypii* contains homologs of all of the *Saccharomyces cerevisiae* genes involved in this pathway. However, in spite of this high degree of conservation *A. gossypii* is not known to have a sexual cycle. We have deleted the main components of this cascade including the pheromone receptor genes *STE2*, *STE3*, the G-protein components encoded by *STE4*, *GPA1*, the MAP-kinase genes *STE11*, *STE7*, *FUS3*, *KSS1* and the transcription factors *STE12* and *TEC1*. The results show that all the *A. gossypii* mutants except *tec1* grew with wild-type rates and sporulated. This differs from *S. cerevisiae* where deletion of any of these genes leads to sterility and in the case of *GPA1* is lethal. Strikingly, deletion of the major transcription factor activated by the cascade, *STE12*, resulted in a hypersporulation phenotype similar to that of an *Agtec1* mutant indicating that the cascade might have a regulatory role in sporulation. To overactivate the pheromone cascade in *A. gossypii* we introduced two point mutations in the MAPKK Ste7 that mimics phosphorylation with the aim to generate a constitutively active Ste7 allele (Ste7DD). Heterologous expression of *Agste7DD* from a strong promoter together with an inducible allele of the scaffold protein AgSte5 in *S. cerevisiae* resulted in a morphologic response. Here we will present the results from our work in *A. gossypii*.

PR1.76

The landmark protein Sec3 links Rho-GTPase signaling and polarized secretion in *Ustilago maydis*

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To establish their polarity, cells have to transport cell wall material to the growing tip. This is achieved by a directional transport and fusion of vesicles with the plasma membrane. A octameric protein complex, the exocyst, marks the areas where active exocytosis takes place. Two proteins of the exocyst complex, Sec3 and Exo70, serve as landmark proteins for exocytosis, whereas the other subunits tether secretory vesicles and mediate their interaction with SNARE proteins. In *S.cerevisiae* it is known that components of the exocyst complex are effector of small GTPases of the Rho family. For example Sec3 interacts with Cdc42 and Rho1 which are involved in the localization and regulation of this protein. We have analyzed the role of small GTPases for the regulation of the exocyst complex in *U. maydis*. In contrast to *S.cerevisiae*, *U. maydis* contains not only Cdc42, but also another small GTPase, Rac1. Since Rac1, but not Cdc42, is critical for polar growth in *U.maydis*, we tested if it is also involved in the regulation of the exocyst complex. We analyzed components of the secretory machinery using genetic, cell biological and biochemical approaches. Our results indicate that Sec3 acts as a critical regulator of polar growth in *U. maydis*. This is consistent with the idea that Sec3 is a landmark protein for polarized secretion. Our biochemical data indicates that Sec3 can interact with Rac1, which supports the idea that Rac1 as a main regulator of polar growth is also involved in the regulation of the secretory machinery.

PR1.77

Golgi localized PI(4)P is required for *Candida albicans* filamentous growth

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Membrane phospholipids, such as phosphoinositide phosphates are minor components of cellular membranes. In eukaryotes, phosphoinositide phosphates play a crucial role in cell polarity and membrane traffic, for example PI(4)P and PI(4,5)P2 are critical for regulating the cytoskeleton. In *Saccharomyces cerevisiae* there are two essential genes encoding PI-4-kinaases, STT4 and PIK1. Stt4 is localized to the plasma membrane and Pik1 is localized to the Golgi. The role of these two phosphoinositide kinases in fungal pathogens remains largely unknown.

We have been examining the function of Pik1 and the distributions of PI(4)P in *Candida albicans* filamentous growth. We have generated strains in which the level of the Pik1 PI-kinase can be manipulated using the Tetracycline repressible promoter system. In semi-permissive conditions, the *pik1* mutants are defective in filamentous growth in both liquid and solid media. Using different fluorescent lipid associated reporters, we have been following the distribution of PI(4)P during the transition form budding to filamentous growth and in various mutants. Our results indicate that distinct pools of PI(4)P at the Golgi and plasma membrane are critical for the yeast to filamentous growth transition. We are currently examining the dynamics of these PI(4)P pools during filamentous growth and the interconnection of PI(4)P and Arf1 proteins in the Golgi.

PR1.78

Conidiation is the major target of LAE1 in *Trichoderma atroviride*

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Trichoderma atroviride (teleomorph *Hypocrea atroviridis*) exhibits a synchronized manner of sporulation in response to light stimulus, and thus serves as a model organism for photomorphogenetic investigations. However, conidiation of *Trichoderma atroviride* strongly depends on carbon source. Here we show the involvement of the LAE1 protein in light sensing by *T. atroviride* and regulation of its development. Phylogenetic analysis shows that LAE1 from *T. atroviride* is orthologous to LAE1 from *T. reesei* (teleomorph *Hypocrea jecorina*) and they both are orthologous to LaeA from *Aspergillus nidulans* (teleomorph *Emericella nidulans*). In the later fungus this protein is the master regulator of secondary metabolite production. A loss of function of *lae1* in *T. atroviride* dramatically reduces sporulation in general and weakens the induction of conidia formation by light. We compare photostimulation of conidiation of *lae1* mutants of *T. atroviride* P1 (*lae1* knockout and *lae1*OE respectively) and the corresponding *lae1* mutants of *T. reesei* by using a broad set of carbon sources in an optimized BIOLOG Phenotype MicroArray. We also show that the two blue light receptor proteins BLR-1 and BLR-2 interact with LAE1 in the developmental regulation of *T. atroviride*. Last not least, the mechanical injury of mycelia, which usually triggers the conidiation in *T. atroviride*, was not functional in *lae1* knockout mutant as compared to parent and *lae1*OE strains. These data suggest that developmental regulation is a main target of LAE1 function in *T. atroviride*.

PR1.79

The formation mechanism of apical sterol rich membrane domains (SRDs) and visualization of SRDs by Photoactivated Localization Microscopy in *Aspergillus nidulans*

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Apical sterol-rich plasma membrane domains (SRDs), which can be viewed using the sterol-binding fluorescent dye filipin, are gaining attention for their important roles in polarized growth of filamentous fungi. The microdomain scaffolding protein flotillin was thought to be a good candidate involved in the formation of SRDs. We analyzed the function of the flotillin orthologue FloA by gene deletion and protein localization in the maintenance of SRDs and polarity. SRDs are known to be necessary for the localization of some components of the growth machinery. To investigate deeply the relation of lipid membrane domains and protein localization, the distribution of microdomains in SRDs are analyzed by super-resolution microscope technique, Photoactivated Localization Microscopy (PALM). Raft membranes and non-raft membranes were visualized by each marker protein tagged with photoconvertible fluorescent protein mEosFP for PALM. The size of SRDs is around a few μm, whereas the size of lipid rafts ranges in general between 10-200 nm. In recent years, super-resolution microscope techniques have been improving and breaking the diffraction limit of conventional light microscopy whose resolution limit is 250 nm. In this method, a lateral image resolution as high as 20 nm will be a powerful tool to investigate membrane microdomains.

PR1.80

High Throughput *in vivo* Footprinting – an improved Method to detect Protein-DNA Interactions

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A prerequisite for understanding the mechanisms responsible for transcriptional regulation in a cell is the knowledge which bases of a promoter region are targeted by proteins during induction or repression of a gene. With that in mind we improved traditional *in vivo* footprinting via LM-PCR [1] to obtain a high throughput technique to detect Protein-DNA interactions. The basis of our enhanced method is fluorescent 5'-[6-FAM]-labeling of DNA fragments and analysis via capillary gel electrophoresis (CGE) [2]. In addition we developed a graphical user interface that is essential in the automatization of data processing and visualization of results.

This improved protocol can be a powerful tool in obtaining a better insight in the regulation of gene expression and supporting the systematic manipulation of expression patterns. Furthermore this high throughput technology allows a detailed analysis of the switching of protein-DNA interaction events in regulons or even genome wide, consequently supporting a better understanding of regulatory networks within an organism, tissue or cell.

The utilization of this refined method has already led to new insights into the *xyr1*-regulon responsible for (hemi-) cellulase-expression in the industrially important fungus *Trichoderma reesei*.

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PR1.81

Functional analysis of silent polyketide synthase genes in *Penicillium chrysogenum*

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A major challenge in the battle against infectious diseases is the resistance of pathogens against the commonly used antibiotics. This urges the need for the discovery of new antimicrobial compounds based on new chemical structures in order to develop a new generation of antibiotics. The functional analysis of cryptic secondary metabolite gene clusters is a promising tool for novel bioactive compounds discovery as many of these gene clusters are not expressed under laboratory conditions.

Polyketides represent a diverse group of bioactive compounds that are widely used as therapeutics due to their antibiotic and cytostatic properties. Polyketides are produced by multifunctional enzymes – polyketide synthases – that are encoded by associated (PKS) genes. Here we report on the functional analysis of (silent) PKS genes in the filamentous fungi *Penicillium chrysogenum*. Sequencing data showed the presence of 20 putative PKS genes in the genome [1], none of which have been characterized before. The expression of most of these genes is silent under laboratory conditions. To activate their expression two strategies are used: i) promoter replacement and ii) activation of the complete biosynthetic pathway through local regulatory genes deletion or overexpression. Strong promoters were chosen from the *P.chrysogenum* genome and used for cloning in the appropriate plasmids. Growth media of strains with expression of silent PKS genes were obtained and analyzed using HPLC and mass spectrometry to reveal the products of these PKS clusters. We will report on the activation of the silent PKS cluster via local regulatory gene expression.

References:

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PR1.82

The NADPH oxidase complex is required for re-orientation of the cytoskeleton during appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

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Recent studies have demonstrated that fungal NADPH oxidases (Nox) play a key role in fungal morphogenesis, cellular differentiation and virulence. In plants, reactive oxygen species (ROS) are implicated in the control of polarised cell growth in root hairs and pollen tubes, and are induced in response to microbial attack. Nox enzymes are flavoenzymes used to generate ROS and have recently been characterised in several eukaryotic organisms including filamentous fungi. How this regulated synthesis of reactive oxygen species by fungal NADPH oxidases plays such an important function in fungal development is, however, not known. Previously we have shown that in the rice blast fungus *Magnaporthe oryzae*, *Mo*Nox1 and *Mo*Nox2 are both independently required for rice blast disease. Here we show that the membrane-bound NADPH complex is necessary for re-organisation of the fungal actin cytoskeleton during plant infection. We demonstrated using Lifeact-RFP gene fusions that the Nox1, Nox2 and NoxR subunits of the complex are independently required for formation of a 5.9 µm diameter actin ring at the base of the specialised appressorium that is necessary for plant infection. Furthermore, we established a further role for Nox in septin ring assembly at the base of the appressorium. We demonstrated using Cdc11-GFP and Chm1-GFP gene fusions that the Nox2 and NoxR subunits of the complex are independently required for initiating phosphorylation of the septin ring and subsequent septin ring assembly. Formation of these sub-cellular structures is an essential prerequisite for the initiation of rice blast disease.

PR1.83

The characterization of the three MEKs codifying by the *Cryphonectria parasitica* genome reveals the importance of a functional Cpkk2 for Cryphonectria hypovirus 1 (CHV1) accumulation

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Cryphonectria parasitica is the causal agent of chestnut blight, a chestnut tree disease controlled by the widespread presence of mycovirus-containing hypovirulent strains. The biological function(s) of *cpkk1*, *cpkk2* and *cpkk3* genes, encoding the three mitogen-activated protein kinase kinases (MEKs) of *Cryphonectria parasitica* were examined through specific knock-out strains and addressed to putative roles in virulence and hypovirus infection. Cpkk1 is the Mkk1-homologue acting in the phosphorylation cascade essential for cell integrity. Cpkk2 is the Ste7-homologue involved in the pheromone responsive pathway, while Cpkk3 is the Pbs2-homologue, the MEK activated during the response to high osmolarity. Our analyses confirmed MEKs to belong to the proper signalling cascade with typical defects in the null mutants already identified for the homologues of phylogenetically related filamentous fungi with some exceptions: abnormal hyphae with a reduced number of septa and thinner cell walls were observed in *Δcpkk1* strain and a stronger defect on growth and development was shown for *cpkk2*-null mutant. The growth impairment did not allow to perform proper mating assays in *Δcpkk1* and *Δcpkk2* strains, which were instead normal for *Δcpkk3*. Virulence on chestnut cuttings was only affected in *cpkk1*- and *cpkk2*-null mutants. A successful CHV1 infection through natural anastomosis with a virus-donor line was obtained in *Δcpkk1* and *Δcpkk3* with common symptoms associated to hypovirus infection. On the contrary, no infection was possible in *Δcpkk2* by anastomosis or transformation with an infectious clone of CHV1, suggesting its important role for maintaining a proper cellular environment for virus replication.