Poster Category 7: Sensing and Responding

PR7.1

Interplay between metabolism and regulation of cell wall structure in *Candida albicans*: application

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The cell wall of the opportunistic human pathogen *Candida albicans* is the point of direct interactions of this fungus with host cells, especially with macrophages. It is a layered structure of polysaccharides. However, how changes in carbohydrate metabolism affect the cell wall composition is not yet understood.

We investigated the effects of the cytochrome *c* reductase inhibitors myxothiazol A and antimycin A on the metabolism and transcriptome of *C. albicans*. Treatment with either inhibitor led to the formation of reactive oxygen species and to fermentative growth, as was visible from changes in the metabolite profile. Transcriptome analysis revealed changes in the expression of genes related to carbon metabolism and stress-response. Moreover, a wide range of cell wall-building genes were differentially expressed. Flow cytometry confirmed changes in the exposure of beta-glucans and mannans, which were in accordance with an increase in phagocytosis of *C. albicans* by murine macrophages.

To gain insight into the regulation of the differentially expressed cell wall genes, computational analysis of a dataset comprising expression data for 5214 genes from 317 different conditions was conducted, focusing on a group of mannosyl-transferase and glycosylase genes. A group of likely regulators contained, for example, the transcription factor Efg1p, which is connected to morphogenesis, carbon metabolism, and resistance to antimycin A. The roles of these regulators in cell wall construction and susceptibility to cytochrome *c* reductase inhibitors were evaluated by gene expression and metabolite analysis of the respective deletion mutants, indicating new connections between regulation of cell wall structure and metabolism.

PR7.2

The bZIP-type transcription factor FlbB: A versatile regulator of *Aspergillus nidulans* asexual development.

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The early stages of asexual development in the model fungus *Aspergillus nidulans* are controlled at the molecular level by a discrete number of regulatory proteins that includes the bZIP-type transcription factor (TF) FlbB. Vegetative hyphae contain two main pools of FlbB, one at the tip and the other at the most apical nucleus. The apical pool requires the interaction with the positional regulator FlbE at or in the proximity of the *Spitzenkörper*. This interaction requires in FlbB a functional bZIP domain, specific central regions and a highly conserved Cys residue. Nuclear FlbB is renewed after each mitotic cycle and under appropriate conditions, activates the cMyb-type TF FlbD. Both factors, in turn, jointly activate the expression of the conidiation-specific TF *brlA*.

A 2D-PAGE screening of proteins in wild type and Δ*flbB* strains showed that the concentration of specific stress-response proteins was controlled through FlbB. *gmcA*, a previously uncharacterized glucose-methanol-choline oxidoreductase coding gene, shows miss-scheduled expression in a Δ*flbB* genetic background and the derived protein is required during development under alkaline pH conditions. Sequencing of mRNA from both vegetative and asexual samples provides for a wide overview on the genes and pathways under the hypothetic transcriptional control of FlbB activity. Preliminary results obtained in the functional characterization of some of these genes are also presented.

Overall, the functional versatility of FlbB provides for a new outlook on morphogenetic change and focuses our future work on the study of the molecular mechanisms through which this TF regulates different cellular processes during development.

PR7.3

Calcipressin – an activator of calcineurin-dependent signal transduction in *Botrytis cinerea*?

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The Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CN) is a conserved protein that plays a critical role in calcium signaling and stress response in eukaryotic cells. CN activity itself is regulated by a class of conserved proteins termed Calcipressins.

Deletion mutants of *bccnA* encoding the catalytic subunit of CN in *B. cinerea* exhibit severe growth and development defects and form small, compact colonies. In comparison, deletion of the only calcipressin homologous gene, *bcrcn1*, affects vegetative growth in a similar way.

While *bccnA* deletion mutants are completely apathogenic, virulence of *bcrcn1* deletion mutants on bean plants appears to be only reduced.

Studies with the inhibitor of CN Cyclosporine A (CsA) showed that the expression of several genes, e.g. those involved in phytotoxin biosynthesis, is regulated by CN and the CN-responsive transcription factor CRZ1 in *B. cinerea*. Interestingly, the same set of genes is down-regulated in the Δ*bcrcn1*mutants indicating that BcRcn1 functions as an activator of CN.

Studies based on fluorescent microscopy showed equal distribution of BcRcn1 in the cytoplasm, but accumulation in close proximity to the nucleus connecting the influence on gene regulation directly with its intracellular localization.

Further characterizations identified motifs in the BcRcn1 sequence probably essential for activation of this regulator and for binding to CN.

PR7.4

Signaling Pathways Involved In Response To Environmental Iron In The Human Fungal Pathogen *Candida Albicans*

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Candida albicans is one of the most frequent fungal pathogens in humans with a compromised immune system. Iron uptake during infection is considered as a virulence factor for *C. albicans*. As host and pathogenic microorganisms are in permanent competition for iron, the host sequesters iron through proteins, which bind this metal ion with high affinity. To overcome this barrier, pathogens have developed different strategies in order to acquire iron from host proteins.

We established a rapid protein extraction method that allows semi-quantitative determination of representatives of multicopper ferroxidases (MCFOs) present in the *C. albicans* cell membrane. Members of this protein family are required for high affinity iron uptake under iron limiting conditions. Removal of ferrous iron from YPD medium by the chelator bathophenanthrolinedisulfonate (BPS) led to induction of MCFOs compared to a control lacking BPS. Moreover, addition of $FeCl_3$ as well as $FeSO_4$ to iron-free RPMI medium (buffered to pH 7.3) induced flocculation of *C. albicans* cells in an iron concentration-dependent manner.

A combination of the iron induced flocculation of cells and the MCFOs protein extraction method mentioned above is used to identify signalling pathways involved in the response to iron availability or drugs interfering with high affinity iron acquisition pathways. This could be accomplished by using different homozygous single gene deletion mutant strains where MCFOs and the flocculation process play the role of “reporters” for reduced or intact response to iron respectively.

PR7.5

Does Fgap1 Regulate *Tri* Gene Expression And Trichothecene B Production In Response To An Oxidative Stress In *Fusarium Graminearum*?

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The filamentous fungus *Fusarium graminearum* infects cereals and corn. It is one of the main causal agent of "Fusarium Head Blight" and "Maize Ear Rot". During infection, it produces mycotoxins belonging to the trichothecenes family which accumulate in the grains. Although the biosynthetic pathway and involvement of the genes (*Tri* genes) have been elucidated, the global regulation of the toxin biosynthesis remains enigmatic. We previously showed that, an H_2O_2 oxidative stress enhances the production of toxins in liquid cultures of *Fusarium graminearum* and increases *Tri* gene expression (Ponts et al, 2007, FEBS Lett 581: 443-447).

In the yeast *Saccharomyces cerevisiae,* the transcription factor Yap1p is required for tolerance to oxidative stress and for the expression of genes coding for detoxification enzymes. In this study, we analysed the role of the corresponding factor in *Fusarium graminearum,* Fgap1, in response to an oxidative stress and its eventual interference with the regulation of trichothecene production. A deleted mutant and a strain expressing a constitutively activated form of the Fgap1 factor in *F. graminearum* PH1 were constructed. To mimic an oxidative stress, we cultured these mutants in GYEP liquid medium supplemented with H_2O_2 or diamid to evaluate their sensitivity and analyse their toxin production. Expression profiles of genes encoding detoxification enzymes controlled by Fgap1 and of genes involved in the biosynthesis of type B trichothecenes were analysed by Q-RT-PCR.

PR7.6

Transcriptional links between light and development in *Neurospora crassa*

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The ascomycete fungus *Neurospora crassa* grows as a branched mycelium with interconnected hyphae during the vegetative phase of its life cycle. Several environmental cues, including blue light, promote a developmental transition that leads to the development of conidiophores and the production of conidia. Several mutants have been isolated that are blocked at different stages of conidiation. One of them, *fluffy*, has been investigated in some detail. The FLUFFY (FL) protein is a 792-aminoacid polypeptide containing a Zn2Cys6 binuclear zinc cluster domain belonging to the Gal4p family. Blue light activates *fl*, and light regulation requires the products of genes *wc-1* and *wc-2* that bind transiently to the promoter of *fl*. The activation by light of key regulatory genes may explain the activation by light of conidiation in *Neurospora*. In *Aspergillus nidulans,* another ascomycete, several genes responsible for the formation of conidiophores are activated by light and their *Neurospora* homologs have been identified in the *Neurospora* genome. We have investigated the regulation by light of these putative regulatory genes in the *Neurospora crassa* wild type and Δ*fl* strains and we have found that deletion of *fl* promotes a light-dependent accumulation of mRNA of some of these putative developmental genes. Our results suggest an interaction between FL and the White-Collar complex in the promoter of light and developmentally regulated genes.

PR.7.7

Shuttling of entire MAPK module from membrane to nuclear envelope links fungal development to secondary metabolism

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The sexual Fus3 MAP kinase module of yeast is highly conserved in eukaryotes and transmits external signals from plasma membrane to nucleus. The module of the filamentous fungus Aspergillus nidulans consisting of the AnFus3 MAP kinase, the upstream kinases AnSte7 and AnSte11 together with AnSte50 adaptor lacks the membrane interacting Ste5 scaffold homolog of yeast. The entire MAPK module interact with each other at the plasma membrane as in yeast. We find a different molecular mechanism how the MAPK signal is transmitted in the filamentous fungus: not only Fus3 but the entire complex of four physically interacting proteins migrates from plasma membrane to nuclear envelope. AnFus3 is the only subunit with the potential to enter the nucleus from the nuclear envelope. AnFus3 phosphorylates the conserved nuclear transcription factor AnSte12 to initiate sexual development and the conserved fungal velvet domain protein VeA to coordinate development with secondary metabolite production. Our data define the nuclear envelope as an additional critical control point for signal delivery of a MAP kinase pathway from the cellular surface through the cytoplasm to target regulators located within the nucleus

PR7.8

Redundant Nuclear Localization Signals Mediate Nuclear Import Of The *Aspergillus nidulans* Transcription Activator Of Nitrogen Metabolic Genes AreA

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The *Aspergillus nidulans* GATA transcription factor AreA activates transcription of genes for uptake and metabolism of nitrogen nutrients. AreA accumulates in the nucleus during nitrogen starvation but not in the presence of nitrogen sources. The AreA protein contains five putative classical SV40 Large T Antigen-type nuclear localization sequences (NLSs) and one putative non-canonical bipartite NLS conserved with mammalian GATA4. We have used two approaches to determine which of these predicted NLSs are functional. First, we fused DNA sequences encoding the putative AreA NLSs to the Green Fluorescent Protein (GFP) gene and introduced these constructs into *A. nidulans*. We determined which of the predicted NLSs are sufficient to direct GFP to the nucleus. UV-fluorescence microscopy showed that the bipartite NLS strongly directs GFP to the nucleus, one of the classical NLSs weakly directs GFP to the nucleus and the other four classical NLSs collaborate to direct GFP to the nucleus. Second, we constructed epitope-tagged gene replacement *areA* mutants affected in individual NLSs and combinations of NLSs to identify by immunofluorescence microscopy sequences required for nuclear localization. Deletion of all five classical NLSs did not affect utilization of nitrogen sources and did not prevent AreA nuclear localization. Mutation of the bipartite NLS conferred inability to utilize alternative nitrogen sources but did not prevent AreA nuclear localization. We determined the effect of this bipartite NLS mutation with combinations of deletions of the five classical NLSs on nuclear localization. Our results indicate redundancy among the AreA NLSs.

PR7.9

Glutamine Synthetase In *Fusarium Fujikuroi* – An Enzyme With Major Impact On The Regulation Of The Secondary Metabolism

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The rice pathogen *Fusarium fujikuroi* produces a broad spectrum of secondary metabolites. The biosynthesis of two of them, gibberellins and bikaverin, is subject to nitrogen metabolite repression, while the mycotoxin fusarin-C is exclusively synthesized with high amounts of nitrogen. Glutamine is regarded as one of the primary effectors in the nitrogen regulation network. The deletion of the glutamine synthetase (GS) – encoding gene *gln1* was performed to deregulate the expression of the nitrogen repressed secondary metabolite gene clusters. To our surprise the deletion lead to complete loss of the expression of the nitrogen repressed as well as nitrogen induced secondary metabolite genes, indicating an additional regulatory function of the GS.

To better understand this regulatory function we studied the expression of the transcription factors AreA and AreB and the ammonium permeases MepB and MepC under different nitrogen conditions, which were all shown to be affected by the GS. To examine whether it is possible to separate the enzymatic and regulatory functions of the GS, we complemented the deletion mutant with *gln1* alleles created by random and side directed mutagenesis. These mutants were screened for the ability to grow without glutamine and produce certain secondary metabolites. In addition, heterologous GS-genes from other species were transformed into the mutant and examined likewise. While in most cases glutamine auxotrophy corresponds with loss of secondary metabolism, in some rare cases the regulatory and enzymatic functions could be separated. The achieved results confirm an important, direct regulatory role of the GS in the nitrogen regulation network.

PR7.10

Light controls *Fusarium fujikuroi* secondary metabolism via distinctive pathways

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Fusarium fujikuroi is best known as a pathogen of rice that causes hyper elongation the plants due to production of gibberellic acids (GAs). Besides GAs, *F. fujikuroi* may also synthesize other natural products such as carotenoids and the mycotoxins fusarin C and bikaverin. Production of these secondary metabolites is influenced by several environmental stimuli including ambient pH and nitrogen availability. Furthermore, the fungal-specific *Velvet*-like complex was shown to affect secondary metabolism in *F. fujikuroi*.

Currently, we focus on investigating light-dependent regulation of secondary metabolism in *F. fujikuroi*. Expression of genes responsible for carotenoid, bikaverin and fusarin C production is regulated by light in distinct ways. Homologous proteins of the *Neurospora crassa* GATA-type transcription factors white collar (WC)-1 and -2 interact in the nucleus and deletion of either encoding gene results in loss of early light-dependent *car* gene induction. Since carotenoid production is maintained in the deletion mutants in constant light, we expected additive effects of additional light sensors. The sequenced *Fusarium* genomes reveal the existence of three cryptochromes/photolyases: Additionally to the genes coding for the class I CPD photolyase Phr1 and the Cry-DASH cryptochrome, *Fusarium* spp. posses a homolog of Phl1 from *Cercospora zeae-maydis* closely related to diatome 6-4 photolyases with regulatory functions. Comparison of *phl1*, *vel1* and WC mutants regarding control of light-dependent secondary metabolites, suggests common and distinct regulatory roles in *F. fujikuroi*. Since carotenoid production is maintained in WC/Phl1 double mutants we postulate a light-independent induction mechanism triggered by reactive oxygen species.

PR7.11

Identification of Novel Interactors of the Trichoderma atroviride Gpr1 7-Transmembrane Receptor

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By transducing extracellular signals to intracellular downstream effector proteins, eukaryotic seven-transmembrane (7-TM) receptors, also known as G protein-coupled receptors (GPCRs), function as sensors. The classical paradigm of 7-TM receptor signalling is based on a physical interaction of the receptor with an intracellular Galpha subunit. In recent years however, evidence accumulated of signal transmission by 7-TM receptors through mechanisms involving intracellular partners other than heterotrimeric G proteins. In fungi, heterotrimeric G proteins are essential for regulating growth, sexual and asexual development, and virulence, but the involvement of 7-TM receptors in these processes remains to be shown for most fungi. Our recent functional characterization of Trichoderma atroviride Gpr1revealed a prominent role of this 7-TM receptor in the antagonistic interaction of the mycoparasite. gpr1-silenced transformants showed an avirulent phenotype probably resulting from their inability to respond to the presence of living host fungi. When analyzing possible interactions between Gpr1 and the three Galpha subunits of T. atroviride, we could not observe any physical interaction between the receptor and Tga1, Tga2 or Tga3. Here we provide the isolation and identification of novel interactors of Gpr1 by screening a T. atroviride cDNA library using the membrane-based split-ubiquitin yeast two-hybrid assay. After selecting for growth on drop-out medium and testing for positive interactions by re-transformation and beta-galactosidase assays, inserts representing 15 different genes could be isolated from the initially obtained 115 colonies. The majority of the encoded proteins were identified to be related to transport processes and ten of them were shown to harbor transmembrane domains.

PR7.12

Conditional expression of the phospho-transmitter gene *ypdA* and the interaction of YpdA with response regulators in *Aspergillus nidulans*.

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Eukaryotic histidine-to-aspartate (His-Asp) phosphorelay systems consist of three types of common signal transducers: His-kinase (HK), a response regulator (RR), and a histidine-containing phosphotransfer intermediate (HPt). In general, HPt acts as an intermediate between HK and RR and is indispensable for inducing appropriate responses to environmental stresses through His-Asp phosphotransfer signaling. Although HPt is thought to be essential among *Aspergillus* species, the molecular mechanism underlying the HPtA essentiality remains unclear. In the present study, we constructed *Aspergillus nidulans* mutant in which expression of the HPt gene *ypdA* is conditionally regulated under the control of the *A. nidulans alcA* promoter (C*ypdA* strain). When C*ypdA* was cultured in CD medium (repressed condition), the transcript level of the *ypdA* gene was decreased to 5% that of the wild type (ABPU1). In CD medium, C*ypdA* showed remarkable growth retardation and formed abnormal hyphae, suggesting that YpdA is an essential component. Downregulation of *ypdA* expression resulted in induction of the *catA* and *gfdB* genes, which are upregulated downstream of the active HogA mitogen-activated protein kinase (MAPK) cascade. We then constructed mutant strain from C*ypdA* by deleting the response regulator gene *srrA* (C*ypdA/ΔsrrA*). When *ypdA* was downregulated, C*ypdA/ΔsrrA* unexpectedly showed more severe growth retardation than the parent C*ypdA*. We are also trying to isolate a mutant from C*ypdA* by deleting another response regulator gene *sskA*. Here, we discuss two-component signaling under the inhibitory conditions of signaling between YpdA and response regulators.

PR7.13

The fungicide fludioxonil induces expression of ABC-transporters Cdr1p and Cdr2p in *Candida albicans* and increases the resistance to fluconazole

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The fungicide fludioxonil is widely used in agriculture, especially to protect grapes and berries from fungal diseases. Residua of this fungicide are occasionally detected in fruits and can therefore be ingested by humans.

The human fungal pathogen *C. albicans* expresses the target of fludioxonil, a type III histidine kinase, called Nik1p. Thus, we investigated the effects of fludioxonil on the growth and transcriptome of *C. albicans*. While inhibition of growth was strongly dependent on the genetic background of the strain, such that growth of the major test strain of this study (SC5314) was hardly affected, we observed a concentration-dependent induction of the expression of the ABC-transporter genes *CDR1* and *CDR2*. The induction of ABC-transporter genes was independent of the presence of the target of fludioxonil, as induction was also observed in a deletion mutant. Deletion of *CDR1* caused a decrease in resistance against fludioxonil, indicating that the fungicide was discharged from the cell by Cdr1p.

The effect of concurrent exposition to fludioxonil and known cargoes of the induced ABC-transporters on the growth of *C. albicans* and the extrusion of the cargo were thus examined. The presence of fludioxonil decreased the export of rhodamine 6G. A synergistic growth inhibitory effect of both compounds was detected. The resistance against fluconazole, however, was increased by fludioxonil. This effect was independent of the presence of Nik1p.
Therefore, it may be concluded that the increase in resistance to fluconazole in the presence of fludioxonil was caused by the induction of the ABC-transporter genes.

PR7.14

The CryA DASH-cryptochrome Of *Fusarium fujikuroi* Is An Active Photoreceptor

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The ascomycete *Fusarium fujikuroi* has a complex secondary metabolism whose regulation has been object of detail attention. In this work we analyze the functional role and biochemical properties of CryA, a DASH-cryptochrome of this fungus that belongs to the photolyase/cryptochrome family. DNA repair and light sensing activities, formerly found in other fungal cry-DASH proteins, have been investigated in CryA. Biochemical data shows that CryA binds the typical cofactors flavin-adenin-dinucleotide (FAD) and methenyl-tetrahydrofolate (MTHF), and that FAD undergoes photoreduction, a previous step required for DNA repair. CryA was able to bind damaged DNA in single and doubled stranded DNA probes. The protein was also able to repair cyclobutane pyrimidine dimers, tested in ss DNA. Regarding its *in vivo* function, *cryA* transcript levels are strongly induced by light, reaching the maximum after 60 minutes of illumination. Δ*cryA* mutants display light-dependent phenotypic alterations when they are grown under nitrogen starvation. Bikaverin biosynthesis is enhanced in illuminated Δ*cryA* liquid cultures, which acquire an intense reddish pigmentation, but this increment is not accompanied by enhanced expression of the specific polyketide synthase gene *bikA*. In addition, these mutants produce a different type of conidia called macroconidia, whose production is rarely detected in the wild type, and which differ from the normal microconidia in size and morphology. Our data suggest that CryA is a light-dependent repressor involved in the regulation of bikaverin production and conidiation. We propose that CryA has a dual function in *F. fujikuroi* as DNA-repair enzyme and photoreceptor.

PR7.15

Transcript response of polyketide synthases from the chickpea pathogen *Ascochyta rabiei* grown under the influence of divalent cations

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Polyketides compounds are natural products that are involved in the development of fungal structures and plant disease. The phytotoxin solanapyrone, a polyketide, has been greatly associated with *Ascochyta rabiei* aggressiveness. The production of solanapyrone in liquid cultures of *A. rabiei* is dependent on the divalent cations calcium, cobalt, copper, manganese, and zinc. Therefore, we hypothesized that certain polyketide synthases (PKSs) in *A. rabiei* may be regulated by these divalent cations. Using bioinformatics, we predicted thirteen PKSs in the genome of *A. rabiei* using fungal-based profile hidden Markov models. Ten of these were predicted as reducing and three as non-reducing. We conducted organic extractions to isolate polyketide compounds from liquid cultures of *A. rabiei* grown under presence or absence of light and/or under the influence of the aforementioned cations. These organic extracts were inoculated on liquid cultures of the single-cell algae *Chlamydomonas reinhardtii* in order to assess their phytotoxic potential; showing that the presence or absence of light during incubation had no effect on phytotoxicity. However, divalent cations added to the liquid media induced the production of phytotoxic compounds. Transcript analysis showed that the PKS genes *ArPKS02*, *ArPKS03*, *ArPKS04*, *ArPKS08*, and *ArPKS09* were expressed only when these five divalent cations were added to the liquid culture. Through BLASTP analysis, ARPKS09 was found to be a homolog to the solanapyrone synthase (*SOL1*) from *Alternaria solani*. Furthermore, all six genes of the *ARPKS09* gene cluster were highly similar and in synteny to the genes of the *SOL1* gene cluster, suggesting horizontal gene transfer.

PR7.16

Production of the polyketide phomenoic acid, by *Leptosphaeria maculans*

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The epipolythiodioxipiperazine, sirodesmin PL, and the polyketide phomenoic acid are secreted in abundance in culture by *Leptosphaeria maculans,* the dothideomycete that causes blackleg of canola. The biosynthetic gene cluster for sirodesmin PL is well-established, but that of phomenoic acid, is unknown. Both molecules have antifungal activity which may allow *L. maculans* to outcompete other fungi in their niche. Domain modelling and comparative genomics with *L. biglobosa* and *Stagonospora nodorum* (neither of which have been reported to make phomenoic acid), was used to predict a candidate polyketide synthase (PKS) for phomenoic acid biosynthesis. A reducing PKS with the following domains was predicted: KS - keto-synthase; AT - acyltransferase; DH - dehydratase; MT- methyltransferase; ER - enoylreductase; KR -ketoreductase; ACP- acyl carrier protein. Of the 14 PKSs in the genome of *L. maculans*, five had reciprocal best hits in *L. biglobosa* 'canadensis' and seven had close matches to PKSs in *S. nodorum*. Seven PKSs had the above domain structure, but only one (PKS2) was highly expressed by *L. maculans* in culture. Expression of PKS2 was considerably reduced by gene silencing in two different genetic backgrounds resulting in significantly reduced levels of phomenoic acid in culture. Four genes flanking PKS2 shared a similar transcriptional profile, suggestive of their being part of the phomenoic acid gene cluster.

PR7.17

G protein-coupled receptors of the human-pathogenic fungus *Aspergillus fumigatus*

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The filamentous fungus *Aspergillus fumigatus* is present in diverse habitats and therefore confronted with a wide variety of environmental stimuli. Due to its ability to grow on numerous nutrients, *A. fumigatus* is also able to colonise the human body. In immunocompromised patients, severe life-threatening infections like invasive aspergillosis can occur, thus making *A. fumigatus* the most important airborne fungal pathogen.

To be able to sense and respond to changing environmental conditions during infection, *A. fumigatus* contains a large array of sensing and signaling mechanisms including G protein-coupled receptors (GPCRs). GPCRs form the largest group of membrane receptors among eukaryotic organisms. Until now, little is known about the stimuli and signal transduction mechanisms of the 15 GPCRs predicted to be encoded by the genome of *A. fumigatus*. Therefore, to understand their impact on fungal growth, development and pathogenicity, it is of major importance to investigate their function in detail and to identify their possible contribution to pathogenicity.

We created a collection comprising single knock-out strains of almost all *A. fumigatus* GPRCs and started to investigate their phenotypes. First results show that some mutant strains show reduced growth and production of conidia, which gave first hints on the importance of functional signaling pathways for survival of *A. fumigatus*. The ongoing analysis will define the function of different GPCRs in a filamentous fungus.

PR7.18

Functional analysis of gene *cutA* of *F. fujikuroi*, encoding a protein of the haloacid dehalogenase family.

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The gibberellin-producing fungus *Fusarium fujikuroi* combines its phytopathogenic capacity with a saprophytic style of life. In nature, the fungi have to survive to changing environmental conditions during their life cycles, and appropriate regulatory networks are necessary to overcome a huge variety of stresses. In this work, we analyze the role and regulation of gene *cutA* of *F. fujikuroi*, orthologous to *cut-1* from *N. crassa*. This gene codes for an enzyme of the haloacid dehalogenase family (HAD), which includes different classes of phosphatases. Targeted deletion of *cutA* results in normal morphology and pigmentation, but reduced capability to grow in osmotic stress conditions. Interestingly, *cutA* is clustered in a head-to-head arrangement with *gldA*, which putatively encodes a glycerol dehydrogenase. *F. fujikuroi* responds to osmotic stress triggering a fast accumulation of glycerol inside the cell, while the strains lacking a functional *cutA* gene are unable to raise the intracellular glycerol content. Both genes seem to be co-regulated, as their mRNA levels increase transiently after heat shock or osmotic stress treatments. In contrast to *N. crassa*, where *cut-1* is down-regulated by light, *cutA* and *gldA* mRNA levels increase after illumination. Mutants of genes *wcoA* and *cryA*, encoding a WC-1 protein and a DASH-cryptochrome, also show this photoresponse, suggesting that these photoreceptors do not participate in *cutA* and *gldA* photoinduction. The stimulation by light of the expression of these genes in *F. fujikuroi* is consistent with a regulatory association between illumination and osmotic stress.

PR7.19

Comparative mRNAs expression patterns between vegetative growth and asexual development in *Aspergillus nidulans*.

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Using the high resolution RNA-Seq, we have analyzed the different mRNA expression patterns between vegetative and asexually developing mycelia of *Aspergillus nidulans*. The pathways and genes that are activated and repressed during vegetative hyphal growth and conidiophore production have been identified. We also describe a substantial number of novel transcripts that are controlled by *U*pstream *D*evelopmental *A*ctivators (*UDA*s, especially FlbB) at different stages of development. Genes involved in secondary metabolism (like polyketide synthases, see poster Rodríguez-Urra *et al*.), increased oxidoreductase activity and/or transcription factors (principally binuclear zinc clusters) are examples of groups regulated by FlbB.

Our analysis indicates that during asexual development the 4% of the transcriptome is modified comparing with vegetative growth, including more genes than previously were anticipated. Of these, FlbB regulates the 60% directly or indirectly. These results may provide a blueprint for further study of the *Aspergillus nidulans* development.

PR7.20

Involvement of saga complex components in transcriptional regulation in *aspergillus nidulans*

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The SAGA complex is highly conserved from yeast to humans. in yeast it is invloved in the regulation of highly regulated genes that respond to environmental stresses, such as metabolic starvation, DNA damage and heat. Bioninformatic and proteomic analyses have shown that the components of the SAGA complex are also present in *Aspergillus nidulans*.

In *A. nidulans*, acetate is a repressing carbon source that leads to similar levels of CreA mediate repression as glucose. *acdX* was identified in a mutation screen in *A. nidulans* to identify genes invloved in acetate repression but not in glucose repression. The conservation of the amino acid sequence of AcdX of *A. nidulans* and Spt8 of *Saccharomyces cerevisiae* suggests that the SAGA complex may have a role in acetate repression in *A. nidulans*, since Spt8 is a component of the SAGA complex.

CreA has been shown to repress the expression of the *alc* regulon, which is required for the ethanol utilization pathway, and two mechanisms have been identified: (i) by the direct repression of the *alcR* gene, which results in almost no expression of the *alcA* and *aldA* genes or (ii) by the mechanism in which CreA represses both the *alcR* gene and the *alcA* gene indepenently of one another.

We report results of experiments undertaken to confirm whether AcdX and/or SptC, which is the *A. nidulans* homologue of the *S. cerevisiae* SAGA complex component Spt3, are invlolved in the transcriptional regulation of the *alc* regulon in glucose or acetate repressing conditions.

PR7.21

Aquaporin and aquaglyceroporins in *Aspergillus nidulans* are dispensable in osmotic stress responses.

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Aquaporin is a water channel protein found in almost all organisms from bacteria to human. More than 200 members of this family were identified up to date. There are two major categories of Major Intrinsic Protein (MIP) channels, aquaporins and glycerol facilitators, which facilitate the diffusion across biological membranes of water or glycerol and other uncharged compounds, respectively. The full genome sequencing of various fungal species revealed that there are 3 to 5 aquaporins in their genome. However, no functional characteristics were studied so far in Aspergillus sp. In *Aspergillus nidulans*, one orthodox aquaporin (*aqpA*) and four aquaglyceroporins (*aqpB~E*) were found in the genome. Knock-out of each aquaporin or aquaplyceroporin didn't show obvious phenotypic change in osmotic stress, suggesting that the function of the genes may be redundant or not be related in osmotic stress responses. However, resistance of fluconazol has been changed in some mutants, indicating that the function of aquaporins play roles in susceptibility of antifungal reagent. [This work was supported by NRF Korea (2009-0072920)]

PR7.22

A Novel Transcriptional Regulator, ClbR, Controls the Expressions of Cellulose- and Hemicellulose-degrading Enzyme Genes by Two Distinct Mechanisms in *Aspergillus aculeatus*

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The cellobiose- and cellulose-induction of the FIII-avicelase (*cbhI*) and FII-carboxymethyl cellulase (*cmc2*) genes were regulated by XlnR-independent pathway in *Aspergillus aculeatus*, which suggests that this fungus possesses an unknown cellulase-genes-activating pathway. To identify the novel *trans*-acting regulatory factors controlling their expressions, we established a positive screening system to monitor the inducibility of the *cbhI* promoter using the orotidine 5'-phosphate decarboxylase gene (*pyrG*) as a reporter. Gene disruption library was constructed by T-DNA insertion using *Agrobacterium tumefaciens*-mediated transformation, and transformants were selected for 5-fluoroorotic acid (5-FOA) resistant under the cellulase-inducing condition. Of the ~6,000 transformants that we screened, one 5-FOA resistant, S4-22, grew poorly on cellulose media and reduced the cellobiose-induced expression of *cbhI*. Southern blot analysis and nucleotide sequence of the flanking regions of the T-DNA inserted in S4-22 indicated that the T-DNA located within the coding region of a putative $Zn(II)_2Cys_6$-transcription factor designated as the cellobiose response regulator (ClbR). Interestingly, the *clbR* disruption resulted in reduced expression of not only *cbhI* and *cmc2* but also genes regulated by XlnR in the presence of cellulose. However, the *clbR* disruption did not affect for XlnR-dependent induction in response to D-xylose and L-arabinose. The *clbR* overexpression led to sustainable cellulase and xylanase production for 10 days, which increased their production by 2- and 5-fold, respectively. These data demonstrate that ClbR participates in cellulose signaling pathway regulated by both the XlnR-dependent and the XlnR-independent pathways.

PR7.23

The plasma membrane receptors Sho1 and Msb2 prime *Ustilago maydis* for biotrophic development

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The dimorphic fungus *Ustilago maydis* switches from budding to hyphal growth on the plant surface. In response to hydrophobicity and hydroxy fatty acids *U. maydis* develops infection structures called appressoria. These structures enable the fungus to penetrate the plant epidermis. After penetration a biotrophic interaction between *U. mayids* and its host plant maize is established. Here we report on the transmembrane proteins Sho1 and Msb2, which are essential for appressorium formation in response to the hydrophobic stimulus. Epistasis analysis revealed that Sho1 and Msb2 act upstream of Kpp2 and Kpp6, two MAP-kinases essential for appressorium formation and plant cuticle penetration, respectively. To unravel the impact of Sho1 and Msb2 on gene regulation we performed genome-wide transcriptional profiling under appressorium-inducing *in vitro* conditions. We found that *sho1* and *msb2* are specifically required for the expression of genes encoding putative secreted proteins. Some of these proteins have been previously shown to be required for the establishment and maintenance of the biotrophic interaction. Our data indicate that Sho1 and Msb2 are sensors of plant surface cues acting upstream of the pathogenicity-related MAP-kinase cascade. This signaling cascade has the capacity to prime *U. maydis* for biotrophic development when hyphae are growing on the plant surface.

PR7.24

Identification of protein kinase A target genes of *Aspergillus fumigatus* by functional genomics

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Aspergillus fumigatus is a saprophytic mould normally inhabiting the soil. The fungus also represents a medically important human pathogen causing severe systemic infections in immunocompromised patients. To survive in these entirely different habitats, the fungus needs mechanisms to sense environmental signals and transduce them intracellularly. One of these signal transduction pathways is the cAMP dependent protein kinase A (PKA) pathway. For *A. fumigatus*, components of this generally well conserved signaling cascade have been characterized in detail and its significance for virulence was shown. To identify target genes of PKA, we performed microarray analyses using a mutant strain overproducing the PKA catalytic subunit in comparison to the corresponding wild type. Following this approach, we were able to find 282 up and 343 down regulated genes involved in different cellular processes like carbon and nitrogen metabolism, cell cycle regulation and ribosome biogenesis. Among these genes potentially regulated by PKA, 23 transcription factors were found of which 21 have been not yet functionally characterized in *A. fumigatus*. 15 of these putative transcriptional regulators were deleted and the mutant phenotypes were characterized under different cultivation conditions. A C6 finger domain protein that shows highest upregulation of all identified transcription factors is located in a potential secondary metabolite gene cluster. Because a gene deletion resulted only in minor phenotypical changes, an overexpression mutant of this transcription factor was created to gain deeper insights into its function.

PR7.25

The *Ustilago maydis* MAP Kinase signaling pathway: Identification of direct MAP kinase targets by phospho-proteomics.

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The basidiomycete fungus *Ustilago maydis* is a plant pathogen that causes smut disease in maize. In this pathogen a conserved mitogen-activated-protein-kinase (MAPK) module regulates important steps of fungal development. We are interested in the identification of direct phosphorylation targets of the related MAP kinases Kpp2 and Kpp6 which control mating and plant colonisation. Currently we are using a phospho-proteomics approach for detecting the phosphorylated targets of these MAP kinases. For this approach we generated strains in which MAP kinase signaling can be induced by expressing a constitutively active version of the MAPKK Fuz7 (Fuz7DD) under an inducible promoter in the presence or absence of *kpp2* and *kpp6*. These strains were phenotypically characterized with respect to formation of conjugation tubes and time-course of activation of remaining MAP kinases. Detection of phosphorylated proteins within complex mixtures was done after Fuz7DD induction by separating proteins by SDS-PAGE and visualizing phosphorylated proteins directly by using the phospho-specific dye Pro-Q Diamond. The current state of these experiments will be described. Differentially phosphorylated proteins, i.e. likely targets of Kpp2 and Kpp6, will be identified by mass spectrometry after enrichment. The role of these proteins in signaling and pathogenicity will then be studied by generating mutants and characterisation of their phenotype. Once these novel targets are characterized we will determine whether related targets are of relevance for disease in the other fungi studied in the ARIADNE network.

PR7.26

Molecular responses of *Trichophyton rubrum* to ambruticin and fludioxonil.

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The incidence of fungal infections is increasing worldwide, mainly due to the high prevalence of immunocompromised patients, promoting the search for new antifungal drugs essential to expand the options of viable therapies. Dermatophytes are one of the most prevalent fungal pathogens that cause cutaneous infections in both healthy and immunocompromised patients. Fludioxonil and ambruticin are antifungals that interfere with the osmoregulation system, leading to hyphal-tip swelling, cell wall alterations, and subsequent cell death. In this work, we present evidence on the efficacy of these drugs on the anthropophilic dermatophyte *Trichophyton rubrum*. The results showed that low concentrations were able to inhibit fungal growth in all evaluated infection models. Moreover, according to the infection site, ambruticin differently modulates the expression of *T. rubrum* genes, showing a site-specific response to antifungal drugs that may reflect the adaptive strategies used by *T. rubrum* to overcome the cytotoxic effects caused by the drug.

PR7.27

Transcriptional Profile of the Dermatophyte *Trichophyton rubrum* in Response to Acriflavin

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The dermatophyte *Trichophyton rubrum* is an anthropophilic fungus that is the most common etiologic agent isolated in cases of human dermatophytoses. Recently, it has become the cause of deep and widespread infections in immunocompromised patients. The therapeutic strategies to control this kind of infection have several limitations such as the appearance of resistant strains and the restricted number of cellular targets. New therapeutic strategies are necessary, being the focus of many investigations. Acriflavin is an acridine derivative compound with antifungal activity involved in topoisomerase inhibition. Although it presents DNA intercalating properties, has been reported the over-expression of genes coding for enzymes involved in mitochondrial respiratory-electron transport and in iron transport, suggesting a broad spectra of cellular effects. In order to better understand its molecular effects we evaluated *T. rubrum* transcriptome in response to Acriflavin in a time-course assay using the next generation sequencing technology SOLiD System. RNAseq was performed comparing *T. rubrum* growth in malt extract medium and the three periods of drug exposure, 3h, 12h, and 24h. The transcriptional analysis revealed 490 modulated genes in response to Acriflavin in the conditions analyzed. These genes are involved in various processes including pathogenicity, transmembrane transport, metal ion binding and fatty acids biosynthesis. Furthermore, several genes involved in glyoxylate cycle, such as malate synthase and isocitrate lyase, considered virulence factors, were repressed by the drug. These genes constitute potential candidate targets for the development of antifungal drugs and reinforce the broad spectrum of effects caused by sub-inhibitory concentrations of acriflavin.

PR7.28

Temperature adaptation in *Rhynchosporium commune*

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Rhynchosporium commune is a haploid ascomycete and a major pathogen of barley (*Hordeum vulgare*). Despite the agricultural and economic importance of *R. commune*, little is known about its evolutionary ecology, including adaptation to different temperatures across populations from climatically diverse locations. We conducted common garden experiments with 126 genetically distinct isolates from 9 field populations, measuring phenotypic variation in growth rates at 12°, 18°C and 22°C.

Populations from colder climates with higher temperature variation grew faster at all three temperatures compared to populations from warmer climates, indicating that the former populations contained more thermal generalists. Across the three temperatures genotype-by-environment interactions (GxE) accounted for 1.3 times more of the phenotypic variance than the genetic variance. Population differentiation for growth rates (Q_{ST}) was significantly lower at 18°C than population differentiation at neutral microsatellite loci (G_{ST}) and not significantly different at 12°C but at 22°C the Q_{ST} was significantly higher, consistent with local adaptation for growth at higher temperatures.

We found that *R. commune* has a high potential to rapidly adapt its growth rate because of the high ratio of phenotypic plasticity to environmentally independent genetic variance. We found that this pathogen with a worldwide distribution has indeed adapted locally to climatic conditions, though not through a shift in temperature optimum but rather by acquiring generally fast growth (in cooler/variable climates) or slow growth (in warmer/constant climates). This latter result implies that there may be costs associated with fast growth under warm/constant climates.

PR7.29

The SOFT homolog PRO40 is part of the cell wall integrity pathway in *Sordaria macrospora*

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The homothallic ascomycet *Sordaria macrospora* has been studied as a model organism for fungal sexual development since the 1950s. In forward genetic approaches several mutants were generated which showed the same sterile phenotype. From this set of mutants it was possible to characterize the PRO-proteins involved in sexual development. One of these proteins is PRO40, a homologue of Soft from *Neurospora crassa*, both are characterized by a WW domain. Several interaction studies revealed MEK1 as an interaction partner of PRO40. MEK1 belongs to one of three MAP-kinase (MAPK) cascades of *Sordaria macrospora*. These cascades are involved in processes like the adjustment of gene expression to changing environmental conditions. They consist of three kinases, which are activated consecutively through phosphorylation. Homology-based analysis puts the MAPKK MEK1 into cell wall integrity pathway. Here, we demonstrate that Δmek1 shows similar growth defects as Δpro40. Beside the interaction of PRO40 and MEK1, there are also hints for a putative function of PRO40 as scaffold protein for the cell wall integrity pathway. Yeast-two-hybrid studies show no direct interaction between MEK1 and the MAPKKK MIK1. In contrast we provide evidence for an interaction between PRO40 and MIK1. To deduce the function of PRO40 in this context further, experiments like co-immunoprecipitation with tagged proteins and generation of constitutively active MAK1 derivatives will be performed.

PR7.30

XlnR-independent Pathway Regulates both Cellulase and Xylanase Genes in *Aspergillus aculeatus*

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To help understand substrate-induced gene expression mechanisms of cellulose- and xylan-degrading enzymes in *A. aculeatus*, factors regulating the transcription of these genes were examined. A comparison of gene expression profiles between the host strain and an *xlnR* disruptant demonstrated that *A. aculeatus* possesses at least two signaling pathways for these genes induction: the XlnR-dependent and -independent pathways. The expression of FI-carboxymethyl cellulase (*cmc1*) and FIb-xylanase (*xynIb*) genes was controlled by XlnR; in contrast, the expression of the FIII-avicelase (*cbhI*), FII-carboxymethyl cellulase (*cmc2*), and FIa-xylanase (*xynIa*) genes was controlled by an XlnR-independent pathway. To gain deeper insight into the XlnR-independent pathway, the expression profile of *cbhI* was analyzed as a representative target gene. Cellobiose together with 1-deoxynojirimycin (DNJ), a glucosidase inhibitor, induced *cbhI* the most efficiently among disaccharides composed of β-glucosidic bonds. Furthermore, cellobiose with DNJ induced the transcription of all genes under the control of the XlnR-independent pathway, whereas genes under the control of XlnR were not induced. GUS reporter fusion analyses of truncated and mutated *cbhI* promoters revealed that three regions were necessary for effective cellulose-induced transcription, all of which contained the conserved sequence 5'-CCGN_2CCN_7G(C/A)-3' within the CeRE, which has been identified as the upstream activating element essential for expression of *eglA* in *A. nidulans*. The data therefore delineate a pathway in which *A. aculeatus* perceives the presence of cellobiose, thereby activating a signaling pathway that drives cellulase and hemicellulase gene expression under the control of the XlnR-independent regulation through CeRE.

PR7.31

Insights into the mechanism for integration of nutrient and light signals in *Trichoderma reesei* (*Hypocrea jecorina*)

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Although the biotechnological workhorse *Trichoderma reesei* (*Hypocrea jecorina*) is predominantly known for its capability of efficient plant cell wall degradation, recent studies show that it has not lost its evolutionary heritage. Adjustment of nutrient utilization and response to light and darkness are achieved by interacting pathways, also under laboratory conditions.

Transmission of nutrient signals via the heterotrimeric G-protein pathway has been shown to be influenced by light. We show that this interconnection is mainly established by the light regulatory protein ENV1 and the phosducin like protein PhLP1, via mutual transcriptional regulation and presumably by influence on GNB1 (G-protein beta subunit) function. ENV1 thereby exerts a more severe effect on gene transcription than BLR1 or BLR2. Lack of either one of the photoreceptors or PhLP1, GNB1 or GNG1 leads to a partial shut down of processes up-regulated in light, indicating that heterotrimeric G-protein signaling exerts its major function in light and is a target of the light response machinery. Consequently, signals transmitted via the G-protein pathway are of different relevance in light and darkness. Investigation of regulation of glycoside hydrolases as one of the major output pathways of this mechanism revealed that 79 % of all genes belonging to this group, representing all GH-families available in *T. reesei*, are potentially responsive to light. We conclude that ENV1 is a key factor in connecting nutrient signaling with light response and establishes a signaling output pathway independent of BLR1 and BLR2.

PR7.32

Signalling the induction of sporulation involves the interaction of two secondary metabolites in *Aspergillus nidulans*

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When growing *Aspergillus nidulans* hyphae encounter the atmosphere they initiate a morphogenetic program leading to the production of spore-bearing structures called conidiophores. Mutants defective in the *fluG* gene fail to initiate asexual sporulation because they lack an endogenous diffusible factor that purportedly accumulates on aerial hyphae, thus signaling development. Culture extracts from a wild type strain can, however, complement this defect when added exogenously. Through a bioassay-guided purification of culture extracts of a wild type strain, a factor that reverted the non-sporulating phenotype of a *DfluG* mutant was purified and identified as dehydroaustinol. This meroterpenoid was only active in fractions containing the orsellinic acid derivative diorcinol. This compound interacts with dehydroaustinol to form an adduct, detected by HRMS in a LC-MS experiment, which prevented dehydroaustinol crystal formation, facilitating its access to the putative receptor. This is, to our knowledge, the first instance in which a signaling compound requires the presence of an assisting molecule to facilitate its mode of action.

PR7.33

The RGS-PX domain containing protein Rgs3 is a novel regulator of the pheromone MAPK signaling pathway in Ustilago maydis

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In the phytopathogenic fungus Ustilago maydis cell fusion of compatible haploid cells is controlled by a pheromone/receptor system. Pheromone triggers the activation of cAMP signaling as well as MAP kinase signaling. We have identified a RGS and PX domain-containing protein termed Rgs3 (Regulator of G protein Signaling), regulating the mating MAPK signaling pathway. Microscopic observation showed that Rgs3 resides in the endoplasmic reticulum. The rgs3 deletion strain showed reduced conjugation tube formation in response to pheromone stimulation, was reduced in cell fusion and in virulence. The activation of the MAP kinase Kpp2 in the rgs3 deletion strain was dramatically attenuated and the induction of b gene expression was also severely reduced upon pheromone stimulation. Moreover, the overexpression of pheromone receptor pra1 in rgs3 deletion strains rescued the defects in Kpp2 activation and conjugation tube formation after pheromone induction. This indicates that there may be a link between Rgs3 and Pra1. Current studies will be presented that at which level Rgs3 regulates the pheromone receptor Pra1.

PR7.34

Functional characterization of light-regulated transcription factors in *Penicillium chrysogenum*

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Many responses to external and internal stimuli have been identified to regulate gene expression in the industrial penicillin producer *Penicillium chrysogenum*. Light for instance acts as a major carrier of information, but in case of *P. chrysogenum* little is known about the effect of illumination on regulatory networks. It has been shown, that light has an effect on morphology and secondary metabolite production, although only few regulators have been found so far at the molecular level. To identify light induced regulatory responses, and the proteins involved, we used microarray-analysis for our experimental approach. We compared expression levels of cultures grown in light with those grown in darkness, to identify differently regulated genes. The putative light regulated genes were compared with sets of genes from previous microarray experiments where expression levels were analysed of disruption strains with deleted genes encoding core elements of the light dependent *velvet* complex [1]. To identify regulatory factors, we screened candidate genes for putative transcription factors by BLAST and PFAM analysis. Four transcription factors, namely PcrpnD, PchoxM, PcatfA and Pc04780 have been selected for functional characterization. Subsequently we have generated deletion strains using the FLP/*FRT* recombination system [2]. Further functional characterisation showed an effect of gene disruption on morphology, conidiation and secondary metabolite production.

[1] Hoff B, et al. (2010) Eukaryot Cell 9: 1236-50
[2] Kopke K, et al. (2010) Appl Environ Microbiol 76: 4664-74

PR7.35

***CoPacC*, pH-responsive transcriptional factor, is involved in the entry mode selection of *Colletotrichum orbiculare* at wounded sites of *Arabidopsis* leaves.**

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Colletotrichum orbiculare (*Co*) is the causal agent of cucumber anthracnose disease. Normally, *Co* forms melanized appressoria which enable the fungus to invade the plant. However, we have recently found that *Co* exhibits hyphal tip-based entry (HTE), uncoupled with melanized appressoria, at wounded sites of *Arabidopsis* leaves. To investigate what kinds of signals at wounded sites induce HTE, we looked for the in vitro conditions that can trigger HTE. As a result, we found that appressorium development of *Co* on hydrophobic surface was suppressed when ambient pH was shifted to alkaline condition. PacC is known to pH-responsive transcriptional factor in several filamentous fungi. To assess the potential involvement of PacC in switching to HTE, we identified the *PacC* homolog of *Co* and generated *CopacC* null mutants. Remarkably, at wounded sites of *Arabidopsis* leaves, the ratio of melanized appressorium formation of the *CopacC* mutants significantly increased in comparison with that of the wild type. Furthermore, the transudate collected from wounded sites of *Arabidopsis* leaves induces HTE-like morphogenesis of *Co* in vitro in the CoPacC-dependent manner, and its activity severely decreased when the transudate pH was altered. It was reported that PalC is the activator of PacC and localizes to cortical structures when pH signalling is active in *Aspergillus nidulans*. To assess the activation of pH signalling during HTE-like morphogenesis and appressorium development of *Co*, we have generated a PalC GFP-tagged mutant. Our results suggest that the PacC-mediated pH sensing and regulation is involved in the entry mode selection of *Co*.

PR7.36

Early Infection of *Ustilago maydis*: Hdp2 and Biz1 Control Appressoria Formation and Plant Surface Penetration

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In the corn smut fungus *Ustilago maydis*, sexual development is initiated by fusion of two haploid sporidia, resulting in a filamentous dikaryon that is capable to infect the plant. This process is orchestrated by the *a* and *b* mating-type loci. The bE/bW transcription factor encoded by the *b*-mating type triggers a regulatory network consisting of different transcription factors e.g. Rbf1, as a master regulator, which is required for the expression of most *b*-regulated genes [1]. To get insight into the processes that precede plant infection, we performed microarray analysis of *U. maydis* cells grown on the plant surface. Two of the genes specifically induced in a pathogenic strain are a C_2H_2 zinc finger transcription factor and a homeodomain transcription factor named *biz1* [2] and *hdp2*, respectively. We show that Δ*hpd2* strains are completely blocked in appressoria formation, whereas Δ*biz1* cells are severely reduced in their ability to form appressoria and to penetrate the plant [2]. Furthermore, Hdp2 appears to be required for the expression of about 30% of all genes induced on the plant surface, while Biz1 can induce about 30% of all genes up-regulated on the plant surface. The loss of regulation of these genes may explain the severe phenotypes observed in Δ*hpd2* and Δ*biz1* strains.

References

[1] Heimel *et al*., 2010; PLoS Pathog. Aug 5;6(8):e1001035
[2] Vraneš *et al.*, 2006; Plant Cell 18: 2369-2387

PR7.37

The global transcription regulator VTA2 of the plant pathogen *Verticillium* spp. mediates biofilm formation and virulence and reprograms yeast for adhesion

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The hybrid phytopathogen *Verticillium longisporum* infects oilseed rape resulting in a servere reduction of oil yield. Little is known about the molecular mechanism of plant host infection, especially the first step when the pathogen interacts and attaches to the host surface before penetration. We have identified genes that are involved in adhesion and fungal biofilm formation by screening a *V. longisporum* cDNA library expressed in non-adherent *S. cerevisiae*. Twenty-four different genes from the cDNA library rescue adhesion in non-adherent yeast. They include two similar cDNAs representing isogenes for the same transcription regulator VTA2 (**V**erticillium **T**ranscription **A**ctivator) which is localized in the fungal nucleus. The genomic loci revealed that the two *V. longisporum* isogenes *VTA2-1* and *VTA2-2* share 96% identity. Both isogenes can be distinguished by the first exon and the third intron of together five exons separated by four introns. *VTA2-1* of *V. longisporum* is identical to *VTA2* of *V. dahliae*, whereas *VTA2-2* is very closely related to *VTA2* of *V. albo-atrum*. Disruption of *VTA2* in *V. dahliae* resulted in loss of biofilm formation and surface hydrophobicity, reduction of virulence on its host combined with defects in morphological differentiation, reduction in growth rate, early maturation of the resting structures (microsclerotia) and sensitivity to oxidative stress. We propose yeast as a model to identify regulator genes of pathogenicity like *VTA2* which is conserved in the genomes of other phytopathogens including *Fusarium*, *Magnaporthe* and *Botrytis.*

PR7.38

Nitrogen source utilization modulates invasive growth of *Fusarium oxysporum* through changes in extracellular pH

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During infection, fungal pathogens activate virulence mechanisms such as host adhesion, penetration and invasive growth. In the vascular wilt fungus *Fusarium oxysporum* the mitogen-activated protein kinase (MAPK) Fmk1 is required for efficient root adhesion, penetration of cellophane membranes and plant infection. Previous work established that MAPK-dependent virulence functions are repressed in the presence of the preferred nitrogen source ammonium, and that this repression can be reversed by rapamycin, a specific inhibitor of the TOR kinase. Here we studied the role of the ammonium permease MepB in ammonium uptake and utilization by *F. oxysporum*, as well as its role in repression of MAPK-regulated virulence function. Mutants lacking MepB or carrying point mutations that affect ammonium transport were still able to perform root adhesion and cellophane penetration in the presence of ammonium. Similarly, deletion mutants in MeaB, a bZIP regulatory protein required for ammonium utilization, are insensitive to ammonium repression of MAPK-dependent virulence functions. In the *F. oxysporum* wild type strain, ammonium uptake from the medium resulted in a rapid decrease in extracellular pH, while this pH shift was abolished in the *meaB* and *mepB* null mutants. Addition of rapamycin did not affect ammonium uptake or the pH shift, suggesting that TOR represses virulence functions through a distinct mechanism. These studies reveal a functional link between ammonium uptake, extracellular pH and MAPK-dependent infectious growth.

PR7.39

The role of the RNA-binding protein Whi3 in control of cell division and development

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In *S. cerevisiae*, the RNA-binding protein Whi3 is required for several processes including cell division and cellular development. Initial studies provided evidence that during cell cycle progression Whi3 functions as a cytoplasmic retention factor for the CDK Cdc28-Cln3 complex. Moreover, Whi3 has been shown to bind a large set of mRNAs *in vivo* indicating that it might be a more global regulator. We have previously shown that Whi3 regulates cellular adhesion by posttranscriptional control of the dual-specificity kinase Yak1, which lies at the center of a signaling pathway for adhesion and stress response. In addition, our current data suggest that Whi3 is able to control adhesion by Yak1-independent mechanisms. We demonstrate that Whi3 controls further regulators of cell adhesion and development at the posttranscriptional level, e.g. the genes for the transcriptional regulators Flo8 and Tec1 and for the G1-specific cyclin Cln1. These data support the view that Whi3 is a more global posttranscriptional regulator for cell growth and development than previously anticipated.

PR7.40

Dynamics of MAPK signaling in *Saccharomyces cerevisiae*

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Integration of multiple signals and execution of the appropriate response are crucial properties of many signal transduction pathways. In *Saccharomyces cerevisiae*, the Fus3/Kss1 mitogen-activated protein kinase (MAPK) module differentially controls the two developmental programs of mating and biofilm formation. For induction of the mating program, a strong pheromone signal is required that strongly activates the MAPK module and leads to the transcription of mating-specific genes. In contrast, activation of the biofilm program and corresponding genes is achieved by stimulation of the MAPK module through a weak signal of unknown nature. Importantly, weak MAPK stimulation does not activate mating and strong stimulation switches off biofilm formation. Previous studies have revealed detailed insights into the topology of the MAPK module. How the dynamic behavior of module components affects the correct processing of different signals is largely unknown. In this study, we aim at understanding the dynamics of the central signaling units and the genetic circuits of the Fus3/Kss1 module. For this purpose, we have established a fluorescence microscopy-based system for quantitative measurement of MAPK signaling *in vivo* at the single cell level. In addition, we are developing mathematical models to identify critical reaction rates and threshold concentrations. These approaches should enable us to identify general regulatory principles of MAPK modules and exploit these insights for the design of synthetic signaling pathways with tailored properties.

PR7.41

A Molecular genetic analysis of the AreA-NmrA interaction in *Aspergillus nidulans*

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The nitrogen metabolite repression system of *Aspergillus nidulans* allows the expression of genes required for the utilisation of non-preferred nitrogen sources to occur only in the absence of the preferred ammonium or glutamine. The transcriptional activation ability of the primary positive regulator of this system, AreA, is modulated through many processes including differential *areA* mRNA stability, regulation of nuclear import/export and competitive binding with the negative acting AreB. AreA function is also modulated by interaction with the co-repressor NmrA and co-activator TamA. The co-repressor NmrA is able to bind to the GATA Zinc-Finger DNA binding region of AreA, but *in vivo* experiments have shown that this binding is not preferential to AreA binding to GATA containing DNA, so the exact mechanism with which NmrA represses the activity of AreA is unknown.
This study aims to investigate this interaction with and repression of AreA through a mutagenic approach. Since overexpression of *nmrA* can prevent the activity of AreA, resulting in an inability to grow on non-preferred nitrogen sources, a screen has been set up to obtain mutants that are insensitive to NmrA activity. The sequence changes in these mutants and the predicted effects on NmrA structure have been determined.

PR7.42

The major changes in the transcriptome during germination occur before isotropic growth and are not affected by the antifungal compound natamycin

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The transcriptome of *Aspergillus niger* was analysed during germination of conidia in the absence or presence of the antifungal compound natamycin. Early germination was accompanied by a reduction in cellular microviscosity and a drop in mannitol and trehalose levels. During 8 hours, normal germlings grew isotropically, followed by polarized growth (germ tube formation) and performed 1 cycle of mitosis. With 3 µM natamycin, germination was arrested at the stage of mitosis and germ tube formation and at 10 µM natamycin conidia even did not swell and accumulated some mannitol.

After 2 h of germination, upregulation in the transcriptome of natamycin treated conidia was very similar to untreated conidia, especially protein synthesis, energy and rRNA translation. Correlation of the RNA profiles between 2- and 8 h indicated that all conidia change extensively, but highest in 10 µM natamycin, despite the lack of morphological change. The RNA profiles of these cells were slightly more similar to dormant conidia then all other samples. After 2- and 8 h, natamycin-treated cells contained increased numbers of expressed genes compared to the controls in a dose-dependent manner. Transcripts of a number of protective compounds specific for dormant conidia were highly accumulated in 8 hour old treated spores. These habits have developed secondary after a large shift in the transcriptome.

All these cues point into the direction that germinating conidia that are confronted with adverse conditions have no focus on strong vegetative development, but regain relatively high stress resistance, absence of growth and prolonged survival.

PR7.43

Analysis of a putative α-carbonic anhydrase from the filamentous ascomycete *Sordaria macrospora*

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Carbonic anhydrases (CA) are ubiquitous enzymes, which catalyze the reversible hydration of carbon and protons. CAs have evolved in all three domains of life. Based on their amino acid sequence and structure, they can be divided into five distinct groups (α,β,γ,δ,ξ) which share no sequence similarity and have supposable evolved independently. All known fungal CAs belong either to the α- or to the β-class (Elleuche and Pöggeler 2010, Microbiology 156: 23-29). The filamentous ascomycete *Sordaria macrospora* encodes four carbonic anhydrases, three of the β-type, termed CAS1, CAS2 and CAS3 and one of the α-type. CAS1, CAs2 and CAS3 have been previously characterized and have been shown to be involved in spore germination, hyphal growth and fruiting-body development (Elleuche and Pöggeler 2009, PloS One: 4:e5177). Here, we show the analysis of the α-CA CAS4. We analyzed the enzyme activity of all four CAs by a yeast complementation experiment.

CAS4 exhibit a signal peptide for secretion. Using Western-Blot analysis we were able to demonstrate secretion of a Flag-tagged version of CAS4. Moreover, using fluorescence microscopy we localized a GFP-tagged-CAS4 in a net-like structure resembling the endoplasmic reticulum. To better understand the role of the *S. macrospora* CAS4 and its interplay with the three β-CAs, we generate a Δcas4 single

PR7.44

Drugs transporters in the fungus *Botrytis cinerea* during infection of treated and non-treated plants

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The grey mould fungus *Botrytis cinerea* causes losses of commercially important fruits, vegetables and ornamentals worldwide. Various fungicides with different modes of action are effective against this pathogen, but isolates with multiple fungicide resistance phenotypes have been observed with increasing frequencies.

In fungi, two major types of membrane proteins participate to drug efflux: ATP binding cassette (ABC) and major facilitator superfamily (MFS) transporters. The activity of these proteins has often been correlated with their gene transcription level, and mutations leading to over-expression of individual genes has been shown to increase export and thereby reduce sensitivity to drugs.

The objective of our work is to identify transporters involved in detoxication during infection of fungicide-treated plants. Gene expression profiling of the *Botrytis cinerea* ABC and MFS transporters genes has been investigated on non-treated and fungicide-treated plants using the Nimblegen microarray technology. First results show that at least 4 ABC transporters and 4 MFS transporters genes are differentially up-regulated, and this is confirmed by PCR analysis. This study aims at a better understanding of the putative role of the fungal efflux system in plant infection as well as in infection of treated plants.