VIp-1

## REGULATION OF FORMATION OF THE INTRACELLULAR BETA-GALACTOSIDASE ACTIVITY IN *ASPERGILLUS NIDULANS*

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Intracellular ß-Galactosidase of the filamentous fungus *Aspergillus nidulans* was not formed during growth on glucose or glycerol, but was rapidly induced during growth on lactose or D-galactose. L-arabinose, and D-xylose also induced ß-galactosidase activity. Addition of glucose to cultures growing on lactose led to a rapid decrease in the ß-galactosidase activity. In contrast, in cultures growing on D-galactose, addition of glucose decreased the activity of ß-galactosidase only slightly. Glucose inhibited the uptake of lactose, but not of D-galactose, and required the carbon catabolite repressor CreA for this. In addition, CreA also repressed the formation of basal levels of ß-galactosidase and partially interfered with the induction of ß-galactosidase by D-galactose, L-arabinose and D-xylose. Phosphorylation of D-galactose was not necessary for induction. Interestingly, a mutant in galactose-1-phosphate uridylyl transferase produced ß-galactosidase activity at a low, constitutive level even on glucose and glycerol, and was no longer inducible by D-galactose, whereas it was still inducible by L-arabinose. We conclude that the biosynthesis of the intracellular ß-galactosidase of *A. nidulans* is regulated by CreA, partially repressed by galactose-1-phosphate uridylyl transferase, and induced by D-galactose and L-arabinose in independent ways.

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VIp-2

## A WD40-REPEAT PROTEIN REGULATES CELL DIFFERENTIATION IN SORDARIA MACROSPORA AND CAN FUNCTIONALLY BE SUBSTITUTED BY A MAMMALIAN HOMOLOGUE

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Fruiting body development in fungi is a complex cellular differentiation process that is controlled by more than 100 developmental genes. Mutants from the filamentous fungus Sordaria macrospora, showing defects in fruiting body formation, are pertinent sources for the identification of components of this multicellular differentiation process. Here, we show that the sterile mutant pro11 carries a defect in the pro11 gene encoding a multimodular WD40-repeat protein. Complementation analysis indicates that the wild-type gene or C-terminal truncated versions of the wild-type protein are able to restore the fertile phenotype in mutant pro11. The subcellular localization of the PRO11 polypeptide was examined using its functional fusion to the EGFP protein. Light microscopic investigation and confocal laser microscopy showed that the fluorescent PRO11-EGFP protein is primarily localized to the cytosol and the membrane. This was confirmed by Western blot analysis, which detected the PRO11 polypeptide in both, the soluble and membrane fraction of the fungal cells.
PRO11 shows significant homology to several vertebrate WD40-proteins, such as striatin or zinedin, which seem to be involved in $Ca^{2+}$-dependent signalling in cells of the central nervous system and are supposed to function as scaffolding proteins linking signalling and eukaryotic endocytosis. Cloning of the mouse cDNA, encoding striatin allowed a functional substitution of the wild-type protein with a restoration of fertility in mutant pro11. Our data strongly suggest that an evolutionary conserved cellular process controlling eukaryotic cell differentiation may regulate fruiting body formation.

## VIp-3

### THE ROLE OF THE ACTIN AND MICROTUBULE CYTOSKELETON IN POLAR GROWTH OF *USTILAGO MAYDIS*

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It emerges that both microtubules and F-actin are involved in polar fungal growth, but details of their relation and cellular tasks are elusive. In this study we focus on the organization and role of microtubules and actin in polar growth of infective hyphae. Expression of a plus-end binding protein Peb1YFP in hyphae, revealed that 85% of the plus ends are oriented towards the growing tip, as well as the basal vacuole, suggesting that plus end-directed kinesins support polar hyphal growth and vacuole formation. Moreover, first indications exist that actin-based mechanisms position the Golgi-apparatus towards the growing tip. Detailed studies, using seven kinesin mutants as well as myosin and dynein mutant strains, are underway to elucidate the detailed role of the cytoskeleton and the corresponding motors in polar growth.

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## VIp-4

### Overlap of nuclear localisation signal and specific DNA binding residues within the zinc finger domain of PacC.

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The transcription factor PacC, mediating regulation of gene expression by ambient pH in Aspergillus nidulans, contains a three zinc finger DNA binding domain (zf-DBD) including a nuclear localisation signal (NLS). We selected, using the “GABA technique”, 38 novel mutations impairing PacC function, of which 21 missense mutations identify individual residues essential for zf-DBD structure/function. PacC repression of gabA (GABA permease) transcription is direct, preventing gabA induction at alkaline pH. A most severe repressing effect is observed in a PacC constitutive background. Reversion of this phenotype can be obtained by affecting PacC DNA binding or its nuclear localisation. We have identified in finger 3 Gln155 that is specifically involved in contacting DNA, while the major role of Lys159 resides in the nuclear localisation of the protein. In contrast, Lys158 is essential both for DNA binding and nuclear localisation. Finger 3 suffices to drive nuclear localisation of GFP, we conclude that it contains an NLS (including Lys158 and Lys159). These residues are within an a-helical basic sequence which is completely conserved amongst zinc fingers of the PacC/RIM101 family and present in an identical position of the last finger a-helix of Drosophila Cubitus interruptus, where it is also involved in nuclear localisation. Our functional analysis agrees with our previous conclusion that finger 1 does not bind DNA and provides in vivo evidence that Trp80 and Trp116, located in the Cys knuckles of zinc fingers 1 and 2, are critical for zf-DBD structure/ function. We propose that PacC and Gli/Ci zf-DBDs belong to a subclass of these domains characterised by possession of a pair of conserved Trp residues involved in the interaction between the two most N-terminal fingers and the presence of an NLS in the $\alpha$-helix of the most C-terminal finger. Loss of PacC nuclear localisation resulting from His142Leu (b-strand) and Phe151Ser (hydrophobic core) substitutions in finger 3 suggests that its folding is required for NLS function. Overlap of DNA binding and NLS may aid release of PacC from its cognate importer(s) upon nuclear translocation, as suggested for zinc binuclear cluster proteins.



**A NOVEL GENE THAT RESCUES A CELL-WALL MUTANT IN ASPERGILLUS NIDULANS**

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Cell walls define the shape of fungal cells and play important roles in mediating many interactions between cells and their environment. The assembly and modification of this complex fabric of polysaccharides and glycoproteins, is incompletely understood. We have identified several mutant strains of the filamentous fungus Aspergillus nidulans, containing defective alleles causing hypersensitivity to the chitin synthase inhibitor Calcofluor White (CFW). CFW hypersensitivity has been tied to cell wall defects in the yeast system (e.g., M. Lussier et al., 1997, Genetics 147:435-450). Using a plasmid genomic DNA library ("AMA NotI", Osherov and May, 2000, Genetics 155: 647-656), we have cloned a genomic fragment that complements on of our mutants (CalC). The plasmid was transposon-tagged and sequenced, and plasmids containing transposons in ORFs were used to re-transform the mutant to determine the ORF responsible for the rescue. The region that rescues the phenotype contains three ORFs, only one of which was PCR-amplified from a representative cDNA collection. The translated product of this ORF has no homology to known proteins; however it contains regions that are similar to a membrane-bound protein. Sequencing of the genomic DNA of the mutant reveals no DNA lesion, so this novel protein is a high-copy suppressor of CalC. Current experiments are underway to make a GFP-fusion protein to monitor its location in the cell.

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**A NEW GENE CHARACTERIZED FROM TRICHODERMA ATROVIRIDE THAT ENCODES AN ENDOCHITINASE WITH ANTIFUNGAL PROPERTIES**

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*Trichoderma atroviride*, a soil-borne filamentous fungus, is a mycoparasite that has been used in biocontrol to inhibit the growth of several serious plant pathogenic fungi. *Trichoderma* produces and secretes a number of hydrolytic enzymes, including chitin-degrading enzymes. These enzymes enable *Trichoderma* to degrade the cell walls of many plant pathogenic fungi and genes encoding them have been studied in detail. We have constructed a degenerate primer that can potentially be used for the isolation of all fungal chitinase genes belonging to chitinase Family 18. This primer was utilized to isolate a new gene from *T. atroviride* P1 encoding a putative endochitinase. The gene contains one short intron and the deduced aa sequence of the spliced ORF shows a protein of 309 aa including a putative signal sequence of 21 aa. The predicted molecular weight of the mature protein is 30 kDa and the gene was hence named *ech30*. Southern analysis indicates that the gene is single copy and, except for its presence in *T. harzianum*, the gene does not exist in other closely related *Trichoderma* species investigated. Best homology was found to an endochitinase from the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum*. Only weak homology was found to other *Trichoderma* chitinases. Real-time RT PCR expression studies reveal that the *ech30* gene is repressed by glucose and induced by the presence of plant pathogenic fungi. Gene expression was studied during direct confrontation assays involving *T. atroviride* P1 isolate by itself (control), *Fusarium culmorum*, *Botrytis cinerea, Rhizoctonia solani* and *Pythium ultimum*. This suggests that ECH30 may be involved in biocontrol.

## VIp-7

### LOCALIZATION OF THE ANTIFUNGAL PROTEIN (AFP) OF ASPERGILLUS GIGANTEUS IN SENSITIVE FUNGI BY ELECTRON MICROSCOPY

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Proteins with antifungal activity have been isolated from differing organisms ranging from bacteria, plants, insects and amphibians to human beings. The antifungal protein (AFP) which is secreted by the imperfect ascomycete Aspergillus giganteus is a small and basic protein which was shown to inhibit the growth of several filamentous fungi, mainly from the genus Fusarium and Aspergillus.
Recently, we were able to show that AFP causes membrane permeabilization in AFP-sensitive fungi, but the underlying mechanism of this action is unknown so far. In the present study we used immunogold staining and transmission electron microscopy (TEM) to localize the protein in AFP-sensitive (A. niger) and -resistant fungi (P. chrysogenum) in order to identify the cellular target site of AFP. Treatment of A. niger and P. chrysogenum resulted in binding of the protein to the fungal cell wall. Remarkably, an accumulation of AFP at distinct areas within the cell wall was only detected for A. niger. This accumulation could be a hint towards a specific binding to some structures within the cell wall which determines the antifungal effect of AFP. In addition, we observed several alterations within the cellular structure of A. niger after AFP treatment.

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## VIp-8

### MODE OF ACTION OF THE ANTIFUNGAL PROTEIN (AFP) OF ASPERGILLUS GIGANTEUS AND ITS APPLICATION IN PREVENTING FUNGAL INFECTIONS ON TOMATO PLANTS

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The antifungal protein (AFP) of Aspergillus giganteus exhibits antifungal activity against a variety of filamentous fungi. It is a highly basic polypeptide consisting of 51 amino acid residues. Four intramolecular disulfide bridges result in an extremely compact structure, making it highly resistant to heat and protease degradation. AFP's growth inhibitory effect is brought about by membrane permeabilization in sensitive fungi. A hydrophobic patch composed of amino acids V30, Y29, Y45 and Y50 as well as a cationic site consisting of lysine residues K9, K10 and K32 may be involved in binding and disruption of fungal membranes. Using chemically modified AFP we could show that the acetylation of lysine residues reduced the antifungal activity significantly. A reduction in growth inhibition was also observed when sensitive fungi were treated with samples of AFP, in which tyrosine residues had been chemically modified by nitrification. From our results we conclude that protein charge and pKa of amino acid residues play an essential part in AFP's mode of action. Interestingly, the disruption of intramolecular disulfide bridges had different effects on fungal sensitivity. Due to its narrow host range and high stability, AFP offers promising potential in the combat of plant and human pathogens. In this regard we could show that Fusarium oxysporum infections of tomato plants can be prevented by the application of AFP.



**MOLECULAR CHARACTERIZATION OF ALTERNARIA BRASSICICOLA FIELD ISOLATES HIGHLY RESISTANT TO DICARBOXIMIDES AND PHENYLPYRROLES**

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Three seed-borne species of the anamorphic genus Alternaria, i.e. A. brassicae, A. brassicicola and A. japonica, are responsible for the blackspot disease, which is one of the most widespread and damaging diseases of some cultivated crucifers. Protection of cruciferous crops against pathogenic Alternaria spp. mainly relies on the use of fungicides. Several families of fungicides are used as seed or foliar treatments, including dicarboximides, carbamates, benzimidazoles and triazoles. Despite this, increasing prevalence of Alternaria blackspot in many countries, and a partial loss of efficacy of some spray programs have been observed. Decline in the control of the disease might be related to several factors, including emergence of fungicide resistance. Screening fungicides against Alternaria species pathogenic to crucifers allowed us to detect A. brassicicola field isolates that expressed high resistance levels (above 100 mg/L) towards both dicarboximides and phenylpyrroles. The molecular basis of dicarboximide resistance is not yet completely understood but several studies with different fungal models have suggested a link between resistance and the regulation of osmotic stress. Hyphal growth tests in the presence of 4% NaCl revealed that the $Dic^{HR}$ –PP $^{HR}$ isolates were slightly more osmosensitive than the fungicide sensitive isolates. Based on the phenotypic similarity between these resistant isolates and some of the Neurospora crassa os-1 mutants, we initiated the cloning of an os-1 histidine kinase (HK) homolog from A. brassicicola using PCR in the presence of degenerated primers. The full-length coding sequence was then obtained by PCR-walking and named AbraHK1. The deduced amino-acid sequence had high homology with other fungal two-component HKs and showed the 6 characteristic N- terminal repeated amino acid domains of ca. 90 amino acids in length followed by a kinase domain and a regulatory domain in the C-terminal part. Comparison of the sequence of AbraHK1 genes from fungicide-sensitive isolates and two fungicide-resistant field isolates (Abra 3 and Abra 40) revealed mutations in the kinase domain for the two latter strains. Single nucleotide substitutions were found resulting in premature stop codon at amino acid position 998 and replacement of Glu by Lys at amino acid position 753 (H-box) for Abra3 and Abra 40, respectively. To our knowledge, this is the first report of fungal field isolates expressing such high levels of resistance to both dicarboximides and phenylpyrroles and carrying mutations outside the six amino acid repeat domains of the two-component HK.

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**REGULATION OF ASYNCHRONOUS MITOSES IN MULTINUCLEATED CELLS**

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We use the filamentous fungus, *Ashbya gossypii,* as a model organism to study the regulation of the nuclear division cycle in multinucleate cells. In this organism, mitosis occurs asynchronously such that neighboring nuclei divide at different times. This is in contrast to most multinucleated cells which generally undergo synchronous mitosis and suggests that *A. gossypii* has a nuclear autonomous cell cycle. Remarkably, this asynchronous mitosis is executed using a similar suite of proteins that drive the budding yeast cell cycle, but without the presence of semi-redundant pairs of proteins produced from the duplication of the yeast genome. This work focuses on understanding how mitotic asynchrony is locally generated and maintained in these cells. We have generated tools to visualize nuclei, spindle pole bodies and the microtubule cytoskeleton in *A. gossypii* cells to analyze the dynamics and regulation of asynchronous mitotic events. Using these tools, we have determined that: 1.) all nuclei have the capacity to divide but do so in asynchrony, 2.) neighboring nuclei exist in different cell cycle stages, and 3.) asynchrony is rapidly restored after a drug induced synchrony arrest. Thus each nucleus has an independent cell cycle and asynchrony is a robust characteristic of this system. We predicted that one way to maintain asynchrony would be to sequester cyclins in nuclei. Surprisingly, while both the G1 cyclins and B-type cyclins are nuclear, they also appear to be present in nuclei at all stages of the cell cycle suggesting orderly cell cycle progression requires a different mechanism than control of cyclin abundance. Given the cyclin localization, the role of CDK/Cyclin inhibitors such as Swe1p and Sic1p may be especially important in this system. Supporting this idea, *swe1* mutants display an increased nuclear density suggesting an increase in the frequency of mitoses. Furthermore, in vivo timelapse data suggests that nuclei pause for an extended time in G2 phase, the period in which Swe1p would be predicted to function. Interestingly, the G1 cyclins are also localized to the tips of hyphae at sites of polarized growth suggesting some link between morphogenesis and this cell cycle. Remarkably, however, polar growth continues even under mitotic arrest conditions leading to hyphae with an extremely low nuclear density. Conversely, mitotic asynchrony persists even in depolarized cells that grow as round spheres rather than hyphae. Thus the nuclear mitotic cycle and polar growth appear to essentially be uncoupled in this organism. When combined, these data describe a novel mode of nuclear division in multinucleated fungal cells.

## VIp-11

### CHARACTERISATION OF KAPA, THE UNIQUE NUCLEAR PROTEIN CARRIER IMPORTIN a IN ASPERGILLUS NIDULANS.

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We have identified for the first time a component of the nuclear protein transport machinery in Aspergillus nidulans. The gene kapA (karyopherin A), encodes for the unique importin a like protein in the Aspergillus nidulans genome. The cDNA sequence of kapA reveals a deduced open reading frame of 553 amino acids with armadillo repeats and high similarity to yeast and human importin a family members. kapA is the unique karyopherin a coding gene in the Aspergillus nidulans genome, as SRP1 is in Saccharomyces cerevisiae, and might be responsible for the nuclear transport of all proteins containing classical nuclear localisation sequences (cNLS). We have identified the transcription factor PacC, that mediates ambient pH regulation in Aspergillus nidulans, as a cargo for KapA. PacC53, the intermediate truncated form of PacC, contains a classical bipartite NLS that is recognised by KapA. PacC53 was found to be preferentially located into the nucleoplasm. Interestingly this cNLS, that is conserved in most of the PacC family members, is removed after further processing to the PacC27 form.
Our 3D structure prediction of KapA suggests an structure/function conservation amongst these nuclear protein carriers. Modelling and molecular data indicate I) a similar auto inhibitory regulatory mechanism in KapA, II) conserved molecular interactions with the importin b1 homologue of A. nidulans, kapB, its counterpart in the mechanism of nuclear transport of cNLS, and III) conserved protein-protein interactions with cNLS.

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## VIp-12

### DISSECTION OF THE RNA SILENCING MECHANISM IN Mucor circinelloides: siRNAs, AMPLIFICATION AND TRANSITIVE SILENCING

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RNA silencing is a sequence-specific RNA degradation mechanism that results in the suppression of gene expression. It is triggered by the deliberate or fortuitous production of double-stranded RNA molecules (dsRNA), which are processed into small sense and antisense RNAs (siRNAs) by the Dicer enzyme. Subsequently, the siRNAs are incorporated into a multiprotein complex, which specifically degrades all mRNA sharing sequence identity with the siRNAs. We have demonstrated the existence of a RNA silencing mechanism in Mucor circinelloides using the carotenogenic gene carB as a visual reporter of silencing. Introduction of non-integrative transgenes containing complete or truncated carB sequences into the wild-type strain induces silencing of the carB function, as a result of the degradation of the carB mRNA. Gene silencing in M. circinelloides is associated with the presence of two size classes of small antisense RNAs, the long 25-nt RNA and the short 21-nt RNA. These two classes of antisense RNAs are differentially accumulated during the vegetative growth of M. circinelloides, the 25-nt class being more abundant at the beginning of vegetative growth, whereas the 21-nt class are predominantly produced late in the vegetative life cycle. The two classes of antisense RNAs do not equally represent all portions of the target gene. Both are preferentially generated from the 3'-region of the carB gene, indicating that the 21/25 nt molecules detected correspond mainly to those resulting from an amplification step. In this step, a RNA-dependent RNA polymerase would produce secondary siRNAs using the target mRNA as a template, being transcription preferentially produced from the 3'-end of the carB mRNA. Secondary 21-nt and 25-nt siRNAs corresponding to sequences of the target gene downstream of the initial triggering molecule can also be detected in silenced transformants, the pattern of accumulation of those secondary siRNAs during the vegetative growth being similar to that described above. These data reveal the existence of spreading of RNA targeting in fungi, a process that has been named transitive silencing. We have also investigated if the two classes of antisense RNAs are processed by two different Dicer enzymes by developing an in vitro assay for Dicer activity. Results obtained using crude extracts of M. circinelloides cells and nuclei-enriched fractions will be presented.

**UPTAKE OF THE PENICILLIUM CHRYSOGENUM ANTIFUNGAL PROTEIN PAF IN SENSITIVE ASPERGILLI**

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The Penicillium chrysogenum antifungal protein PAF exhibits growth inhibitory properties against opportunistic human and plant pathogenic filamentous fungi. So far, the effects of this secreted, highly basic and cysteine- rich protein were found to be multifactorial [1].
Ultrastructural studies by transmission electron microscopy revealed the formation of microvesicular-like structures between the plasma membrane and the cell wall and an irregular mitochondrial morphology in PAF- treated hyphae.
In order to identify the site of action of PAF, indirect immunofluorescence studies were performed. PAF-sensitive Aspergillus species revealed a specific intracellular localisation of the protein. The uptake of PAF was found to be temperature- and energy- dependent. In the presence of inhibitors of the electron flow in cytochrome oxidase, namely 2.5 mM sodium azide ($NaN_3$) or potassium cyanide (KCN), no intracellular fluorescence was detectable. Similar results were obtained using 100 µM carbonyl cyanide m-chlorophenylhydrazone (CCCP), an uncoupler of oxidative phosphorylation. In the presence of latrunculin B, a specific inhibitor of actin polymerisation and of endocytosis, the uptake of the antifungal protein was inhibited and PAF accumulated in the outer cell layers, indicating that active internalization is a prerequisite for protein activity [2].
This work was partially supported by the Austrian Science Foundation, grant FWF P15261 to F. M. and by the Austrian National Bank grant ÖNB 9861.

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[2] Oberparleiter C., Kaiserer L., Haas H., Ladurner P., Andratsch M. and Marx F. (2003). Active internalization of the Penicillium chrysogenum antifungal protein PAF in sensitive Aspergilli. Antimicrob. Agents Chemother., Nov., p. 3598-3601

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**IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF POLYUBIQUITIN GENES FROM ASPERGILLUS NIGER**

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The morphology of citric acid production strains of the filamentous fungus, Aspergillus niger is sensitive to a variety of factors including the concentration of manganese ($Mn^{2+}$). We are interested in studying the genes associated with morphology formation in A. niger during citric acid production or non citric acid production growth. Here we report the cloning of two clones that correspond to genes ubi1 and ubi4. The ubi1 gene encodes a carboxyl extension protein of ubiquitin and the ubi4 encodes four head-to-tail repeats of the ubiquitin sequence. Both ubi1 and ubi4 were induced during non-citric acid production growth of A. niger. When A. niger was grown under conditions promoting citric acid production, the transcription of both ubi1 and ubi4 remained relatively constant. However, when A. niger was shifted to filamentous growth via the addition of $Mn^{2+}$ the transcription of the ubi4 gene dramatically decreased within 40 min, while the transcription of the ubi1 gene remained relatively constant. Under filamentous growth conditions, the transcription of the ubi4 gene was relatively low on the first day, significantly increased after two days growth, and remained relatively constant thereafter. On the other hand, the transcription level of the ubi1 gene was relatively high on the first day, then dramatically decreased and remained at a low level Thereafter. The ubi4 gene was further characterized via homologous replacement and promoter analysis. A 0.97 kb promoter fragment was isolated and inserted upstream of the b-glucuronidase reporter gene. The promoter activity and its responses to different growth conditions were examined via a b-glucuronidase activity assay. The ubi4 gene homologous replacement cassette was introduced into A. niger via **Agrobacterium tumefaciens**-mediated transformation. The effects of $Mn^{2+}$ and other growth conditions on the transgenic strains will be reported. The results of this study will provide us further insight into the role of ubi1 and ubi4 genes associated with morphology formation in A. niger.

## VIp-15

### ENRICHMENT OF ISOLATED SEPTAL PORE CAPS OF THE PLANT PATHOGEN RHIZOCTONIA SOLANI

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The septal pore caps (SPCs) are situated at both sides of the dolipore septum within intact hyphae of many Basidiomycetous fungi. Though ultrastructural studies on SPCs demonstrated different SPC morphologies (tubular, non-perforate and perforate), the function of the SPC is still not well understood. In this study, we used the plant pathogen Rhizoctonia solani as a model organism for its large perforate SPCs (about 1.6 – 2.0 μm). To characterize the SPC we used three different approaches. Firstly, we used a number of fluorescent biomarkers (among others Calcofluor white, WGA-alexa488 and ER-tracker) to visualize the SPC, which resulted in different patterns of staining of the SPC region, illustrating the different biochemical properties of the SPC. These biomarkers can be used to monitor the fate of the SPC during the isolation methods. Secondly, we were able to isolate and enrich SPCs after French pressure cell press fractionation, isopycnic and differential centrifugation. Fractions were processed and analyzed with a transmission electron microscope, showing that not only the SPC was enriched, but also the plugging material that remained attached to the SPC (Fig.1). Thirdly, we used laser microdissection to isolate the dolipore septum region from 1 μm sections of hyphae. Protein analysis of the enriched SPC fraction and the microdissected regions will give us more insight in the protein content of the SPC and the plugging material. Once the proteins are known, a better understanding of the SPC function can be achieved.

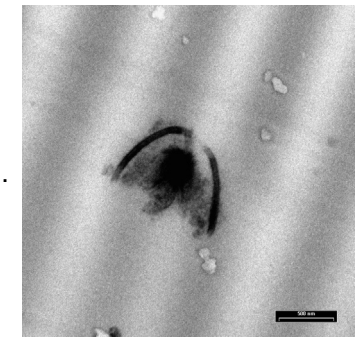

Figure 1: Transmission electron micrograph of an isolated SPC of Rhizoctonia solani, showing the SPC, the plugging material and the connecting fibrillar network

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## VIp-16

### SUPPRESSION OF NEUROSPORA CRASSA COT-1 MORPHOLOGY BY ENVIRONMENTAL STRESSES AND FARNESOL - A QUORUM-SENSING SIGNAL MOLECULE

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Neurospora crassa grows by forming spreading colonies. cot-1 is a colonial temperature-sensitive mutant that ceases to elongate and produces multiple branches at the restrictive temperature. The presence of increased medium osmoticum (NaCl or sorbitol), H2O2, ethanol or reduced availability of fermentable carbon sources significantly suppressed the cot-1 phenotype and was accompanied by altered levels of cAMP-dependent protein kinase (PKA) activity. The cot-1 phenotype could also be partially suppressed by direct inhibition of PKA with KT-5720. When grown at 10-fold cell density (10x6 conidia/ml), cot-1 exhibited near-wild type morphology (at restrictive temperatures), indicating that a biotic-derived environmental signal can phenocopy the effect of abiotic stresses. Replacing 60% of low density cell medium with spent medium obtained from high density cultures of cot-1 or wild type abrogated the cot-1 hyperbranching phenotype. Similarly, culturing cot-1 in the presence of 40-70 micromolar farnesol (a compound shown to be involved in Candida albicans Quorum sensing) suppressed the cot-1 phenotype. High pressure liquid chromatography coupled with photo diode array spectral analysis and spiking with commercial farnesol confirmed that N. crassa produces and secretes farnesol into the growth medium in a cell density-dependent manner. Our results suggest that COT1 is involved in environmental stress response and Quorum sensing, and that altering PKA activity bypasses the requirement for a fully functional COT1.

**A RNA-BINDING PROTEIN FROM *USTILAGO MAYDIS* THAT MOVES IN PARTICLES ALONG MICROTUBULES IS INVOLVED IN FILAMENTATION**

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Pathogenic development in Ustilago maydis is accompanied by a morphological transition from sporidial to hyphal growth. Since in higher eukaryotes RNA-binding proteins play an essential role during developmental processes, we deleted genes encoding putative RNA-binding proteins in U.maydis. One of these encodes Rrm4, containing three N terminal domains with sequence similarity to the RNA recognition motif and a C terminal PAPC domain known as protein interaction interface. The corresponding deletion strain is impaired in filament formation and a Rrm4-Rfp fusion protein is moving as particles along microtubuli labelled with Gfp-Tub1. Thus, we hypothesize that Rrm4 is part of a ribunucleoportein complex transporting RNA to the hyphal tip, a process apparently essential to support fast growth. To isolate target RNAs that are specifically recognized by Rrm4 in vivo we performed a yeast three-hybrid screen. We identified a putative response element located in the 3' untranslated region of a small open reading frame of unknown function. Rrm4 specifically recognizes this sequence via its RRM domains. Currently, we are focusing on unravelling the molecular composition and function of RNA containing Rrm4 particles in U. maydis.

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**INTRASPECIFIC MOLECULAR DIVERSITY OF GLOMUS MOSSEAE ISOLATES FROM DIFFERENT GEOGRAPHIC ORIGIN**

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Arbuscular mycorrhizal (AM) fungi are key microorganisms of the soil-plant system that are fundamental for soil fertility and plant nutrition. Ecological studies of AM fungi are hampered by difficulties in morphological and molecular identification. In particular, the discriminant power of different molecular methods is reduced by the limited knowledge of the range of variation at species and sub-species levels.
To test the genetic variability of the AM fungal species Glomus mosseae, we analysed eight isolates originating from different geographic areas: North America (Arizona, Florida, Indiana), Africa (Namibia), Northern Europe (England) and Mediterranean Basin (Italy, Syria).
Polymorphisms were revealed by PCR-RFLP of internal transcribed spacer (ITS) of ribosomal DNA and by direct amplification of mini and microsatellite regions. Genomic DNA of the isolates was amplified by using primers designed on M13, microsatellites (GTG)5 and (GACA)4. ITS region was amplified, using a heminested protocol, with two pairs of universal primers (ITS1F-ITS4 and ITS1-ITS4), which yielded a fragment approximately 580 bp long. The PCR fragment was then digested with restriction enzymes TaqI, DpnII, HinfI, HphI and RsaI.
Depending on the restriction enzyme used, groups of isolates with common RFLP patterns were detected. On the contrary, M13 and microsatellites primers gave a different level of resolution when compared with ITS, since each isolate was characterized by a distinctive pattern. The amplified ITS fragments of the eight isolates were then cloned and sequenced.

## VIp-19

### MAINTENANCE OF ASYNCHRONOUS MITOTSIS IN MULTINUCLEATED A. GOSSYPII CELLS

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A. gossypii, a filamentous, multinucleated fungus, is unique in that mitosis is asynchronous within a single cytoplasm. In these cells, neighboring nuclei are in different cell cycle stages, unlike most multinucleated cells. Therefore we speculate that novel modes of cell cycle regulation may be key to limiting mitosis in time and space in A. gossypii cells. As a basis for this study, we have identified homologues of nearly all known yeast cell cycle genes.
The experiments presented here examine how a cell establishes and maintains a nuclear autonomous cell cycle. One possible mechanism is through nuclear sequestration of cell cycle control elements. Thus, one approach we are taking is to force key cell cycle proteins out of the nucleus to assay if this leads to synchronous mitosis. If nuclear sequestration is the basis for asynchrony this raises the question of how and if newly translated proteins find the nucleus from which they originated, which would also be an important aspect of maintenance of asynchrony. Using the protein-DNA interaction of the GFP-Lac repressor and Lac operator system, we are investigating whether different, nuclear-localized cell cycle proteins are always reentering their transcriptional mother nucleus. Another possible mechanism to maintain insulation between the different nuclei, is to specifically control nuclear import. Therefore the dependency of the localization of different cell cycle proteins on various karyopherins is an additional part of this work.
While these experiments allow us to examine mechanisms of nuclear insulation, we were also interested in the function of conserved cell cycle regulators in asynchronous mitosis. Therefore we generated a series of mutant strains in which either the B-type cyclins or the regulatory protein SIC1 were deleted. Surprisingly the deletion phenotypes often revealed a much more severe phenotype than the deletion of the homologue genes in S. cerevisiae. This result suggests that in contrast to S. cerevisiae, cyclin genes appear not to be functionally redundant but rather each is specialized and essential for normal morphogenesis and nuclear division in A. gossypii. We hope that these studies bring novel insight into mitotic control in eukaryotic, multinucleated cells, such as tumor cells.

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## VIp-20

### FUNGAL SPORES IN SPACE SIMULATION EXPERIMENTS

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Meteorites could be natural vehicles for transporting resistant life forms, such as fungal or bacterial spores, through space. With the international project SPORES, experimental indications will be provided to the question whether and to what extent meteorite material offers enough protection against the harsh environment of space for spores to survive a long-term stay in space. The spores will be exposed to space conditions on the outside of the ISS for three years. After return to earth the survival of the spores and alterations of their DNA will be examined. Presently ground simulation experiments (Experiment Verification Tests) at the space simulation facilities of the German Aerospace Center (DLR) in Cologne with the fungi Trichoderma koningii and Penicillium italicum are carried out. The parameters vacuum, UV radiation and temperature were tested. Germination of both fungal spores was not affected by vacuum. Even after radiation with 1000 J/m_, both fungi survived, at least some of the spores. On the molecular level (single-spore cultures of fungi) AFLP analysis showed changes in the DNA after UV radiation and vacuum. Fungal spores survived periods of high temperature (60° C), but this tolerance definitely depends on the duration of the impact. Both organisms were tolerant against ultra-low temperature (-80° C) with an interruption (+20° C for 1 day). They also survived repeated oscillation around 0° C. Vacuum conditions improved the survival rate of both fungi at high temperature and oscillation around 0° C. This phenomenon might be due to the absence of oxygen. In order to clarify this hypothesis the experiment was repeated in an argon atmosphere. Under argon the survival of the fungal spores was even better than under vacuum. Therefore the absence of oxygen seems to be one reason for the higher tolerance of the spores under vacuum conditions. Future simulation experiments, in which the parameters UV radiation and vacuum are combined, will show whether this 'vacuum effect' also results in a higher resistance of the spores against UV radiation. The protective function of artificial meteorites will be examined as well.

**LOCALIZATION OF GLUCOAMYLASE EXPRESSION IN ASPERGILLUS NIGER**

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It was shown that only a restricted number of hyphae at the periphery of the colony secrete glucoamylase (Gla) (Wösten et al., 1991). We here studied whether this is due to regulation at the transcriptional level. Strains of Aspergillus niger expressing GFP under regulation of the homologous inducible glaA promoter or the constitutive gpdA promoter of Aspergillus nidulans were grown for 5 days on xylose medium. After inducing the glaA promoter by transferring the colonies for 6-8 hours to medium containing maltose, GFP fluorescence was studied by confocal microscopy. Fluorescence was quantified by measuring the mean pixel values of selected hyphae. Data was analysed with the statistical program SPSS. Variation of fluorescence was significantly larger when GFP was expressed behind the glaA promoter when compared to gpdA driven expression. Two groups of hyphae could be distinguished, those expressing Gla highly and those that express this gene only to a low level. Interestingly, the branches belong to the latter group. Similar results were obtained with both strains when GFP fluorescence was studied in 1-day old germlings grown on maltose. These data indicate that differential secretion of Gla occurs at the transcriptional level. Currently we are confirming these results with in situ hybridizations and with destabilized GFP variants.

Wösten et al. (1991) J Gen Microbiol 137, 2017-2023.

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**INTRA-ISOLATE DIVERSITY IN GLOMUS MOSSEAE, DETECTED BY VEGETATIVE COMPATIBILITY TESTS AND MOLECULAR ANALYSES.**

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Molecular methods utilized for genetic diversity studies of arbuscular mycorrhizal (AM) fungi have often detected varibility within species. Intersporal and intrasporal polymorphism was revealed by Internal Transcribed Spacer (ITS) analysis, Amplified Fragment Length Polymorphism (AFLP) and M13-primed PCR profiles on Glomus spp. and Gigaspora spp.
In this work, a polyphasic approach has been adopted with the aim of studying diversity among five different lines of a G. mosseae isolate originated from Rothamsted collection (yellow vacuolate strain), maintained for about 20 years in different European collections: Pisa and Torino (Italy), Granada and Barcelona (Spain), Dijon (France).
Poor information is available on the occurrence of diversification between these lines, although a slight genetic variability was shown on three of them by random amplified polymorphic DNA (RAPD) analysis and microsatellite-primed PCR.
The characterisation of our lines involved two steps:
molecular analysis, carried out by using internal transcribed spacers restriction analysis (ITS-RFLP), RAPD analysis, M13 minisatellite and microsatellite-primed PCR;
biological analysis, performed by means of vegetative compatibility tests.

Reproducible RAPD profiles were obtained after performing an optimisation procedure on DNA template amount, amplification mix composition and thermal cycle programme for each of the primer OP_A used. Vegetative compatibility tests were carried out by microscope-aid assessment of anastomosis occurrence on paired germinated spores belonging to different lines and by DAPI staining and viability analysis. Results obtained from molecular analyses showed high similarity between cultures. Vegetative compatibility tests showed that the frequencies of perfect anastomosis were always significantly lower when mycelia belonging to different fungal lines were paired, with respect to intra-line pairings.
In the absence of perfect anastomosis, different hyphal interactions were detected, ranging from contact without interference to incompatible response. Incompatibility reactions, occurring either before or after hyphal fusions, were characterized by protoplasm withdrawal from the tip of the approaching hypha and septa formation.

## VIp-23

### CHITIN SYNTHASES ARE A PUTATIVE CARGO OF MYO5, A CLASS V MYOSIN IN USTILAGO MAYDIS

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Class V myosin motors utilize F-actin to support intracellular traffic in eukaryotic cells. In *U. maydis* a class V myosin (Myo5) is involved in polar growth and pathogenicity. Interestingly, Myo5 mutants are hypersensitive to the chitin synthase inhibitor Nikkomycin Z, suggesting that chitin synthases are a cargo of Myo5. In order to gain further support for this notion, we constructed GFP fusion proteins to all chitin synthases encoded by the genome of *U. maydis*. Four CHS-GFP fusion proteins localized towards the growing bud tip and the hyphal apex, suggesting that they are putative cargos of Myo5. In agreement, their localization was insensitive to microtubule disruption by Benomyl, but strongly affected by the actin drug Latrunculin A. When expressed in a conditional Myo5 mutant, one chitin synthase was mislocalized at restrictive conditions, but became repolarized after 3 h. This repolarization was actin-dependent, suggesting that other myosin transporters can substitute for Myo5. Our data suggest an interaction of Myo5 and a subset of chitin synthases.

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## VIp-24

### FUNCTIONAL ANAYSIS OF CLPT1P, A RAB/GTPASE FROM THE BEAN PATHOGEN COLLETOTRICHUM LINDEMUTHIANUM

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Colletotrichum lindemuthianum, a responsible agent of bean anthracnose, secretes extracellular proteins notably cell wall degrading enzymes both in vitro and in the host plant. The production of these enzymes can be controlled during not only their synthesis but also the process of intracellular transport. Rab/GTPases, members of the Ras superfamily, play an important role in regulation of vesicular transport. Recently we have identified a Rab/GTPase encoded gene from C. lindemuthianum. This gene is later called CLPT1 for C. lindemuthianum protein transport1. CLPT1 is able to complement the yeast thermosensitive allele sec4-8, indicating that the CLPT1 encoded protein functions, as yeast Sec4p, in the transport of secretion vesicles from Golgi Apparatus to the plasma membrane ( Dumas et al., Gene (2001) 272: 219-225 ). To investigate the role of CLPT1, we chose to express a dominant-negative allele of CLPT1 in C. lindemuthianum. This strategy consisted to substitute a codon Asn123, located in the nucleotide phosphate binding site, by a codon Ile. The resulted protein can block the activation cycle of native protein. The dominant-negative allele was placed under the control of an inducible promoter of C. lindemuthianum endopolygalacturonase CLPG2, which confers a transient induction of gene when the fungus was cultured in presence of pectin or during the appressorial formation. Different mutants were obtained, named N123I and analyzed. Although these mutant strains showed a normal morphological development, a remarkable reduction of extracellular pectinases was measured while they were grown in presence of pectin. Further more, cytological analysis, by means of electron microscopy, revealed an aberrant accumulation of secretion vesicles. In addition, decrease of pathogenicity was observed. These mutants showed the essential role of CLPT1 in secretion traffic relating to the production of extracellular pectinases and to the pathogenicity of C. lindemuthianum.

**CONIDIAL ANASTOMOSIS IN *NEUROSPORA CRASSA***

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Using live-cell imaging, we have analysed the process of hyphal homing and fusion (anastomosis) between conidial germlings of *Neurospora crassa* labelled with different vital dyes and GFP probes. Specialised, morphologically distinct hyphae (called conidial anastomoses tubes [CATs]) are produced by conidia > 4 h following hydration. In wild type strains, CATs are thinner (2.72 ± 0.6 μm) than germ tubes (3.52 ± 0.3μm). Conidial anastomosis tubes grow towards each other, and reorientate themselves back towards each other if they are moved relative to each other with laser tweezers. This provides clear evidence for the existence of, as yet unknown, diffusible chemotropic signals being involved in the homing response of CATs. In contrast to 'fusion hyphae' which undergo fusion in the centre of a mature colony, CATs do not undergo branching. Fusion between CATs is independent of mating type, and occurs between conidial germlings of the same and different mating types. Further morphological, physiological and genetic markers are currently being used to further characterize CATs and the role that these structures play in the life cycle of N. crassa.

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**CLASS I AND II CHITIN SYNTHASE GENES, CHSC AND CHSA, OF ASPERGILLUS NIDULANS ARE REQUIRED FOR NORMAL SEPTUM FORMATION AND TRANSCRIPTIONAL INDUCTION OF DEVELOPMENTAL REGULATOR GENES BRLA AND ABAA.**

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chsA and chsC of Aspergillus nidulans encode class II and I chitin synthases, respectively. Currently, we have several lines of evidence showing that delta chsA delta chsC double mutants (delta AC mutants) are defective in cell wall integrity. They are also defective in asexual development: a striking reduction in the number of conidiophores and aberrant conidiophore morphology. Here, we employed electron and fluorescence microscopy to investigate the cell wall of a delta AC mutant in detail. In the lateral cell wall of the delta AC mutant, electron-transparent regions were thickened. Septa of the delta AC mutant were aberrantly thick and had a large pore. Some septa were closely located to adjacent septa, and nuclei were irregularly distributed in the delta AC mutant hyphae. We also found that the mRNAs of brlA and abaA, regulatory genes for conidiation, did not accumulate under conidiation-inducing conditions in the delta AC mutant. Collectively, our data indicate that both chsA and chsC are cooperatively involved in normal cell wall construction and proper septum formation, which may be a prerequisite for the accumulation of brlA and abaA mRNAs to induce normal conidiation.

## VIp-27

### A. GOSSYPII HOMOLOGUES OF S. CEREVISIAE BUD GENES INFLUENCE BRANCHING PATTERN AND POSSIBLY SEPTUM BIOGENESIS

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Polar growth is a fundamental process in filamentous fungi and is necessary for disease initiation in many pathogenic systems. The ability to establish and maintain cell polarity is fundamental for morphogenesis and development of unicellular and multicellular organisms. Many genes have been identified in S. cerevisiae involved in polarized growth. Homology searches with the genome sequence of the filamentous fungus A. gossypii have revealed homologues of the S. cerevisiae genes: ScRAX1, ScRAX2, ScBUD7, ScBUD8, ScBUD9. These genes encode putative transmembrane proteins. In S. cerevisiae they are crucial for the bipolar budding pattern observed in diploid cells but play no role for the axial budding pattern in haploid cells. We are interested in determining the role of AgRax1p, AgRax2p, AgBud7p and AgBud8p in the filamentous growth pattern of A. gossypii. Therefore, we generated deletions and GFP fusions in these genes by PCR-mediated gene targeting. In contrast to A. gossypii wild type cells, in Agrax1, Agrax2 and Agbud8 deletions up to four branches can simultaneously emerge from the main hypha, and additional new branches are formed between the already developed ones. Tip growth of the main hypha is markedly reduced when branches start growing. An altered branching was not observed in the Agbud7 deletion. AgRax2-GFP localizes at the tips of hypha and at the septum where it appears just after actomyosin ring formation, and before chitin deposition. AgBud7-GFP is found in dot-like structures which oscillate. The observed structures are able to divide keeping a similar density throughout the hyphae. We will also present results of complementation of S. cerevisiae gene deletions with A. gossypii homologues.

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## VIp-28

### NEWER INSIGHTS INTO HEXA, THE MAJOR PROTEIN OF WORONIN BODY IN ASPERGILLUS ORYZAE

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Woronin bodies acting as plugs to seal septal pores protect the fungus in response to hyphal damage by preventing the loss of cytoplasmic constituents. Although Hex1 protein from Neurospora crassa is well characterized, the molecular mechanisms involved in the assembly of Hex1 and signals that trigger the plugging of septal pore by Woronin body remain unclear. Aspergillus oryzae hexA gene homologous to hex1, was earlier characterized and 2 mRNA transcripts, the spliced (shexA) and nonspliced (nshexA) were reported. In the present study the GST-sHexA fusion protein was expressed in Escherichia coli. While the antibodies raised against sHexA recognized both the spliced (_19 kDa) and non-spliced (□24 kDa) forms of HexA in the wild strain, no HexA forms were detected in the hexA disruptant strain of A. oryzae. Search for functional sites on HexA revealed the presence of a putative protein kinase C (PKC) phosphorylable “serine” residue within a putative hydrophobic calmodulin-binding domain (CaMBD; LFSKIKAAFEDGHGSVRALVIN) at its C-terminal end. In vitro binding assay supported by calmodulin overlay assay and calmodulin affinity chromatography confirmed the calmodulin binding nature of HexA. In addition, the 5’ upstream region of hexA contained 4 STRE (Stress Response Element), 3 CDRE (Calcineurin Dependent Regulatory Element) and one RLM1 (PKC1-regulated transcription factor) putative consensus motif sequences that are known to have regulatory function in cell wall repair mechanisms in Saccharomyces cerevisiae. We hypothesize a coordinated role of calmodulin binding and/or phosphorylation by PKC in the regulation of disassembly/assembly of HexA and septal pore plugging by Woronin body. In order to verify the relevance of these domains in Woronin body function, efforts to mutate the putative PKC phosphorylable site [S151A] and delete the CaMBD are under way.

**ISOLATION AND FUNCTIONAL ANALYSIS OF AOVAM3, A PUTATIVE HOMOLOGUE OF YEAST VAM3 AND PEP12 IN FILAMENTOUS FUNGI, ASPERGILLUS ORYZAE**

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Two unique characteristic features that differentiate the vacuolar system of filamentous fungi from that of the budding yeast are the existence of tubular vacuole-like structures and the presence of only one homologue gene among the post-Golgi syntaxins, PEP12 and VAM3. Such intriguing observations in addition to the industrial importance of the koji mold, A. oryzae, prompted us to isolate and characterize the VAM3 homologue gene (Aovam3) in A. oryzae. AoVam3p showed 25% and 27% homology to Vam3p and Pep12p, respectively. Further, Aovam3 cDNA complemented the phenotypes of both vam3 and pep12 null mutants, indicating that AoVam3p is a functional homologue of Vam3p and Pep12p. Since efforts to construct an Aovam3-disrupted strain proved unsuccessful, an Aovam3 conditional expression strain, TPVII, was constructed. TPVII failed to develop aerial hyphae under Aovam3-repressed condition. This phenotype was restored by the expression of EGFP-AoVam3 fusion protein. The EGFP-AoVam3 fluorescence was observed on the membranes of large developed vacuoles and tubular vacuole-like structures. Furthermore, fusion of large vacuoles and formation of tubular vacuole-like structure between vacuoles was visualized by time-lapse imaging.

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**NATURAL ANTISENSE TRANSCRIPTS (NATS) IN AGARICUS BISPORUS, CHARACTERISTICS AND QUANTIFICATION OF EXPRESSION**

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Natural antisense transcripts (NATs) have been shown to have a regulatory role in gene expression in prokaryotes, but the few reported examples of eukaryotic NATs are of individual genes. Recently six up-regulated genes of the cultivated mushroom (A.bisporus) have been shown to have antisense transcripts detected by independent techniques of sequencing, Northern hybridisation and quantitative PCR (TaqMan). The genes complemented by the NATs encode proteins with diverse functions. This finding of six NATs suggests that natural antisense transcripts are widespread. They are characterised by long length, polyadenylation at 3' end, perfectly complementary sequence and contain no introns or open reading frames. These natural antisense transcripts are therefore distinct from small RNAs (short interfering (si)RNA, micro (mi)RNA or short temporal (st)RNA). Antisense transcript levels of the genes have been quantified using TaqMan PCR. The percentage of antisense to sense ratio of the six genes was found to vary greatly (4.1 - 660%). Statistical analyses reveal that for one gene the antisense/sense ratio is constant over time but is distinctly different between tissues of the A.bisporus fruitbody, and for another gene the converse was shown. It appears therefore that the extent of antisense transcription is controlled at the individual gene level. Three possible mechanisms for the origin of antisense have been described (1) transcription of the opposite strand of the gene (converging promoters), (2) rearranged loci, or (3) transcription of sense mRNA by a RNA-dependent RNA polymerase (RdRp). The sequence characteristics of the antisense suggest sense transcription by RdRp. Some sense strands contain a putative RdRp binding motif in the 3' untranslated region. We hypothesize that the partitioning of sense transcription between transcripts containing or not containing this 3' motif may regulate the level of antisense synthesis.

## VIp-31

### EXPRESSION OF THE RED FLUORESCENT PROTEIN DSRED-EXPRESS IN FILAMENTOUS ASCOMYCETE FUNGI

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The recently reported red fluorescent protein DsRed from the reef coral *Discosoma* sp. represents a new marker that has been codon-optimized for high expression in mammalian cells. To facilitate expression of DsRed in ascomycete fungi, we used the clone pDsRed-Express (Clontech) for constructing a plasmid vector, pPgpd-DsRed, containing the constitutive *Aspergillus nidulans* glyceraldehyde-3-phosphate (*gpd*) promoter. This vector was used for co-transformation of *Penicillium paxilli*, *Trichoderma harzianum* and *T. virens* (syn. *Gliocladium virens*) together with either pAN7-1 or gGFP, both containing a gene for hygromycin resistance for transformant selection. In addition, gGFP contains a green fluorescent protein (GFP) gene previously expressed in Ascomycetes. Expression of DsRed-Express was obtained in all three fungi, indicating that DsRed can be used as a highly effective vital marker in Ascomycetes. Dual marked transformants expressed both DsRed-Express and GFP in the same mycelium and was used for non-quantitative comparison of the intensity of the fluorescence using confocal laser scanning microscopy (CLSM).
The characteristics of DsRed make it an ideal candidate for fluorescence imaging and will be useful for multicolour experiments together with GFP. Thus, when combining different antagonistic fungi for studying their effect, the monitoring of each strain is now possible for differently labelled strains. The GFP variants, emitting blue, cyan and yellow light, has overlapping emission spectra, which means that CLSM and equivalent equipment is necessary for proper discrimination. In contrast, conventional fluorescence microscopy is sufficient to distinguish between co-expressed DsRed-Express and GFP, because of the considerable difference of the spectral properties of DsRed and GFP. Since the *gpd* promoter is known to function in a large number of Ascomycetes, we expect that our construct will have general utility for DsRed-Express expression in additional species.

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## VIp-32

### GENETIC INTERACTIONS BETWEEN RAS AND RHO-TYPE GTPASES COORDINATELY CONTROL CELL POLARITY IN PENICILLIUM MARNEFFEI

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The establishment and maintenance of cell polarity is a fundamental aspect of developmental programs and cellular differentiation. *Penicillium marneffei* is an emerging fungal pathogen of humans which is capable of alternating between a multicellular filamentous and a unicellular yeast growth form, a process known as dimorphic switching, in response to an extrinsic stimulus (temperature), At 25˚C *P. marneffei* grows vegetatively as multinucleate filamentous hyphae which can undergo asexual development (conidiation) to produce complex cellular structures which culminate in the formation of asexual spores (conidia). At 37˚C growth occurs as uninucleate yeast cells which divide by fission and which represent the pathogenic form. Little is known about the molecular events involved in the establishment and maintenance of the developmental states in *P. marneffei* and the control of the dimorphic switching process. We have used *P. marneffei* as a model to examine the molecular mechanisms which control cell polarity and have characterised the roles a number of small GTPases from *P. marneffei*.

Ras and Rho GTPases have been examined in a wide variety of eukaryotes and play varied and often overlapping roles in cell polarisation and development. Studies in S. cerevisiae and mammalian cells have defined some of the central activities of these GTPases however these paradigms do not explain the role of these proteins in all eukaryotes. Unlike yeast, but like more complex eukaryotes, filamentous fungi have Rac-like proteins in addition to Ras and CDC42. To investigate the unique functions of these proteins and determine how they interact to co-ordinately regulate development we undertook a genetic analysis of GTPase function by generating double mutants of the Rho GTPases cflA and cflB and the newly isolated Ras GTPase rasA from the dimorphic pathogenic fungus, P. marneffei. P. marneffei growth at 25˚C is as multinucleate, septate, branched hyphae which are capable of undergoing asexual development (conidiation), while at 37˚C, uninucleate pathogenic yeast cells which divide by fission are produced. We have shown that RasA (RAS) acts upstream of CflA (CDC42) to regulate germination of spores and polarised growth of both hyphal and yeast cells. In addition, the gasC gene, encoding a heterotrimeric G protein a-subunit is a key regulator of the initiation of polarised growth. CflA and CflB (RAC) coordinately control hyphal cell polarisation despite CflA and CflB having unique roles in regulating conidial germination and polarised growth of yeast cells (CflA) and polarised growth of conidiophore cell types and hyphal branching (CflB). There is no apparent control of CflB by RasA.

**The AgBNI1 Formin Homology Protein is essential for hyphal formation in Ashbya gossypii**

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Formin Homology Proteins are known nucleators of actin cables in many different organisms. Here we present data for AgBNI1 a Formin Homology Protein from Ashbya gossypii. AgBNI1 is essential for hyphal formation and might be involved in dichotomous branching as shown by deletion mutants and activated alleles. Deletion mutants of AgBNI1 lack an intact actin cytoskeleton and do not form mature hyphae. In contrast, cells carrying the activated allele show tip-branching prior to emeregence of lateral branches, which is never observed in the wildtype.
In addition we identified four different members of the family of small Rho-type GTPases that are capable of binding to the AgBNI1 protein and therefore might be regulators of the latter. To further investigate the role of these members of the Rho-family we integrated activated alleles into the genome of Ashbya gossypii.
However no single activated rho-allele was capable to mimic the phenotype of an activated AgBNI1 indicating that either several different GTPases or other so far unknown factors are necessary for activation of AgBNI1.