

# Workshop IV

Signal Transduction

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The *pal* ambient pH signalling pathway is a novel signal transduction pathway made up of six components (PalA, B, C, F, H and I), as determined by thorough classical genetic analysis carried out in *Aspergillus nidulans*. The *pal* pathway signals ambient alkaline pH to PacC, a zinc finger transcription factor mediating regulation of gene expression by ambient pH.

While the actual mechanism by which pH signalling results in PacC activation is not understood, it appears that the PalB cysteine protease catalyses the first of the two proteolytic processing steps of PacC, necessary for activation of this transcription factor. PalH and PalI localise to the plasma membrane, in agreement with computer predictions of transmembrane domains, and are likely candidates for ambient pH sensor(s). The PalC sequence gives no clue as to its possible function, but its presence would appear to be restricted to filamentous fungi, whose cells display a characteristically polarised mode of growth and are coenocytic. PalA appears to be connected to endocytosis. PalA is a homologue of *S. cerevisiae* Bro1p/Vps31p, a class E Vps protein involved in the multivesicular body pathway. In agreement, PalA, like its yeast homologues Bro1p and Rim20p, interacts with Vps32p. Of note, two-hybrid analyses have shown that Vps32p also interacts with Rim13p, the yeast PalB cysteine protease homologue. PalA and its mammalian apoptosis-involved homologue AIP1/Alix bind a tetrapeptide consensus YPXL/I motif. Two such motifs are present in PacC and have been shown to have a physiological role. Therefore, PalA could help recruit the PalB protease and its PacC substrate to the multivesicular body. An attractive speculation linking incomplete pieces of information is that pH signalling involves activated receptor endocytosis, triggering PalB activity at the surface of the multivesicular body.

**IVo-2****TWO G ALPHA SUBUNITS AND A MAP KINASE ARE INVOLVED IN HOST RECOGNITION AND MYCOPARASITISM-RELATED GENE EXPRESSION IN TRICHODERMA ATROVIRIDE**

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Mycoparasitic species of *Trichoderma* are commercially applied as biological control agents against various fungal pathogens. The mycoparasitic interaction is host specific and includes recognition, attack and subsequent penetration and killing of the host. Investigations on the underlying events revealed that *Trichoderma* responds to multiple signals from the host (e.g. lectins or other ligands such as low molecular weight components released from the host's cell wall) and host attack is accompanied by morphological changes and the secretion of hydrolytic enzymes and antibiotics. Degradation of the cell wall of the host fungus is – besides glucanases and proteases – mainly achieved by chitinases. In vivo studies showed that the *ech42* gene (encoding endochitinase 42) is expressed before physical contact of *Trichoderma* with its host, probably representing one of the earliest events in mycoparasitism, whereas Nag1 (N-acetylglucosaminidase) plays a key role in the general induction of the chitinolytic enzyme system of *T. atroviride*. Investigations on the responsible signal transduction pathways of *T. atroviride* led to the isolation of several genes encoding key components of the cAMP and MAP kinase signaling pathways, as alpha and beta subunits of heterotrimeric G proteins, the regulatory subunit of cAMP-dependent protein kinase, adenylate cyclase, and three MAP kinases. Analysis of knockout mutants, generated by *Agrobacterium*-mediated transformation, revealed that at least two alpha-subunits of heterotrimeric G proteins as well as a MAP kinase are participating in mycoparasitism-related signal transduction. Two G alpha subunits were shown to be involved in sporulation, secretion of extracellular enzymes, production of toxic secondary metabolites, and the formation of morphological changes like coiling around the host hyphae, whereas knockout of the *tmk1* MAP kinase-encoding gene revealed its involvement in attack and overgrowth of the host fungus and light-induced sporulation.



## DIFFERENTIAL TRANSCRIPT DEADENYLATION MEDIATES NITROGEN AND NITRATE SIGNALLING IN ASPERGILLUS NIDULANS

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Nitrogen metabolism in *Aspergillus nidulans* is subject to regulation by the GATA transcription factor AreA, the activity of which is determined by the quality and quantity of the available nitrogen sources. One mechanism that modulates AreA activity involves 3'UTR-dependent degradation of the areA transcript under nitrogen repression conditions, triggered by accelerated deadenylation of the mRNA signalled by glutamine (Gln) or ammonia (1-3). Here we report that a similar mechanism acts to regulate the expression of the nitrate and nitrite reductase structural genes, *niaD* and *niiA* respectively. Furthermore, the *niaD* and *niiA* transcripts are significantly more stable in the presence of intracellular nitrate ( $\text{NO}_3^-$ ). The 5'UTR of the *niaD* transcript is sufficient to modulate transcript stability in response to these two physiological signals. The  $\text{NO}_3^-$  stabilisation effect is dominant to Gln-dependent destabilisation, intracellular  $\text{NO}_3^-$  stabilising the *niaD* transcript even under nitrogen repressed conditions. Both Gln-mediated degradation and  $\text{NO}_3^-$ -dependent stabilisation of the *niaD* transcript is regulated at the level of deadenylation. Poly(A) shortening of the *niaD* transcript is triggered upon the addition of Gln whereas intracellular  $\text{NO}_3^-$  inhibits deadenylation of the 3' end regardless of the presence of Gln. Intriguingly, transcript stability is also involved in the regulation of a number of other genes. Here we present evidence that expression of an ammonium (*MepA*) and a nitrate transporters (*NrtB*) and also a second GATA transcription factor involved in nitrogen metabolite signalling, *AreB*, are all regulated such that their respective transcripts are more labile in the presence of high intracellular Gln concentrations. Regulated transcript stability may therefore represent a major regulatory mechanism in *Aspergillus nidulans* and other fungi.

- 1) A. Platt, T. et al. (1996) EMBO J. **11**:2791-2801
- 2) I. Y. Morozov et al. (2000): Mol. Microbiol. **37**:1248-1257
- 3) I.Y. Morozov et al. (2001). Mol. Microbiol. **42**:269-277

## IVo-4

### SMALL GTPASES OF THE RHO/RAC FAMILY REGULATE CYTOKINESIS AND CELL POLARITY IN USTILAGO MAYDIS

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Small GTPases form a family of highly conserved proteins that play important roles in the spatio-temporal regulation of many cellular processes. They act as molecular switches and trigger downstream effectors. In the dimorphic basidiomycete *Ustilago maydis*, the small GTPases *Cdc42* and *Rac1* regulate cytokinesis and morphology.

We were able to generate viable *cdc42* deletion mutants which display a cell separation defect, but are rather unaffected in cellular morphology. This implies that *Cdc42* is part of a signalling cascade that regulates cytokinesis in *U. maydis* by triggering formation of a secondary septum. We had previously identified the Rho/Rac specific GEF *Don1* and the protein kinase *Don3* that act in the same signalling pathway.

In contrast to the well studied model organisms *S. cerevisiae* and *S. pombe*, *U. maydis* contains also a *Rac1* homologue, which is highly similar to *Cdc42*. A *rac1* deletion mutant showed an unexpected phenotype. Whereas wild type *U. maydis* cells grow vegetatively by budding, *rac1* mutant cells propagate in a fission-yeast like manner. *Rac1* and *Cdc42* share at least one essential function since a double mutant is nonviable. Surprisingly, overexpression of wild type *Rac1* results in a strong polarization of cells leading to extensive filamentation.

These results indicate that the related small GTPases *Rac1* and *Cdc42* exert distinct functions in *U. maydis*. Thus, *U. maydis* can serve as a model organism to study the functions of *Rac1* and *Cdc42* at the molecular level. We are curren



**CHARACTERIZATION OF THE STRESS RESPONSE IN TRICHODERMA HARZIANUM: ROLE OF HOG KINASE AND cAMP PATHWAYS.**

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Trichoderma harzianum strains are cosmopolitan soil-borne fungi, able to antagonize phytopathogenic fungus and to develop symbiotic interactions with plants, that results in positive effects, similar to the ones exerted by some mycorrhiza-. In soil, T. harzianum is exposed to a highly variable environment with significant changes affecting, temperature, pH, oxygen, and water and nutrient availability. Many of these changes occur during Trichoderma life cycle, triggering specific stress responses to external factors. In some cases, stress-related responses can also be detected in Trichoderma strains when attacking other fungal cells or during root penetration. These changes (i.e. in water potential and substrate availability) have to be sensed by the fungal cell to adapt its metabolism and develop the appropriate response.

Stress response has been extensively studied in *Saccharomyces cerevisiae*, where it is controlled by the Hog-pathway. Hog1 is a MAP kinase protein which regulates a number of transcriptional regulators, such as Msn2/Msn4, which is involved not only in response to water potential stress (hyperosmotic stress) but also to temperature, acid pH and oxidative stress, or nutrient starvation. This pathway acts in opposition to a cyclic AMP pathway in regulating stress gene expression. We have performed two different analyses. First, we have cloned the *hogT* and *pkaR* genes, homologues to the MAPK *hog1* and the regulatory subunit of protein kinase A *pka1* in T harzianum CECT 2413. We have also analysed the phosphorylation state of HogT under different conditions and its relationship with mycoparasitism and plant interactions. We are developing the construction and functional analysis of null mutants. On the other hand, we have carried out membrane-array hybridization of an EST library using RNA obtained from mycelia growing in glucose, chitin, chitin plus cAMP, chitin plus anisomycin (a HOG pathway activator) or chitin plus H89 (a PKA inhibitor), as well as RNA obtained from different mutant strains. From these gene expression profiling data, a picture of the genes induced under stress conditions, and the potential co-expression in antagonistic and plant-fungus interactions can be established.

**REGULATION OF CONIDIAL GERMINATION AND GROWTH IN ASPERGILLUS FUMIGATUS BY A MITOGEN-ACTIVATED PROTEIN KINASE THAT SENSES NITROGEN,**

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Mitogen activated protein kinases (MAPK) play key roles in regulating cellular responses to extracellular signals. The signals transduced by MAPKs ultimately alter patterns of gene expression that lead to a physiological adaptation in response to the extracellular stimulus. In fungi one such MAPK pathway is the one that senses the osmolarity of surrounding medium. This signal transduction pathway has been variably called the hog, for high osmolarity glycerol, or stress activated kinase pathway. In phytopathogenic fungi this MAK has been implicated in plant pathogenesis (ref). Because of this we wanted to determine the role that this MAPK might play in pathogenesis in the human pathogenic fungus *Aspergillus fumigatus*. We have determined that the components of this MAPK system are conserved in this opportunistic human pathogenic fungus, and the transcriptional response of components of this signal transduction pathway is also conserved. Using a deletion mutant of the MAPK gene *sakA*, we found that this MAPK negatively regulates conidial germination in response to the nitrogen source in the medium. We also found that *sakA* transcription is activated by starvation for nitrogen or carbon during vegetative growth. These observations implicate the SAKA MAPK signaling system in nutritional sensing and growth regulation, a previously unknown function for this MAPK. Finally, we determined that the *sakA* deletion mutant does not display any defect in virulence in a murine model of invasive pulmonary aspergillosis.

