V
CELL BIOLOGY AND SIGNAL TRANSDUCTION

Chair:

Nick Read & Joseph Strauss

Vo-1

Time-lapse imaging of mitotic regulatory proteins of *Aspergillus nidulans*

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A gamma-tubulin mutation of *Aspergillus nidulans* affects the regulation of mitotic progression independent of microtubule nucleation (Prigozhina *et al.*, 2004, Mol. Biol. Cell 15:1374-1386). We hypothesize that this may involve altered localization of mitotic regulatory proteins to the spindle pole body (SPB). In collaboration with the laboratories of Dr. Michael Hynes and Dr. Stephen Osmani, we have developed a rapid and efficient gene targeting procedure for *A. nidulans* that allows us to create GFP-tagged proteins easily (Genetics, in press). We have created C-terminal GFP-tagged versions of a number of proteins including the *A. nidulans* homologs of the mitotic regulatory proteins Mad2, Cyclin B, Cdc14 and Mps1. As a first step in determining if their distribution is altered by gamma-tubulin mutations, we have begun to image them in a wild-type background. In each case the tagged gene is functional and is controlled by its own promoter. In interphase, Cdc14 is in the nucleoplasm and at the SPB, but (unlike in some other organisms) it is largely absent from the nucleolus. As nuclei enter mitosis, Cdc14 disperses, and then reappears at the SPBs in anaphase. It disappears from the SPB late in mitosis, but reappears at forming septa minutes later. Cyclin B is visible in the nucleoplasm and on the SPB of G2 nuclei. As nuclei enter mitosis, it is briefly located at the SPBs and in the nucleoplasm, apparently on chromatin. It disappears rapidly first from the SPBs and then from the chromatin and is hard to detect by anaphase. Mad2 is found in multiple dots at the nuclear envelope in interphase. As nuclei enter mitosis it moves briefly to the SPB then is seen along the spindle. The nuclear envelope dots re-form as the nuclei exit mitosis. Mps1 localizes to the SPB in interphase but disappears from the SPB in mitosis.

Supported by grant GM31837 from the National Institutes of Health.

The COP9 signalosome is essential for development and oxidative stress response

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The multiprotein complex COP9 signalosome (CSN) of the filamentous fungus *Aspergillus nidulans* resembles the mammalian CSN and is essential for multicellular development. Six of the fungal CSN subunits interact in the yeast two-hybrid system, and the complete eight-subunit CSN (CsnA - CsnH) was purified by a functional fusion of subunit 5 (CsnE / Jab1) to a codon-optimized TAP* tag. No complex can be purified from *csnA* or *csnD* deletion strains, and strains deleted for different subunits (A, B, D, E or A/B) all display aberrant red coloring and a block in sexual development. The intact complex is thus essential for CSN function. Proteome analyses revealed that a *?csnE* strain fails to induce proper oxidative stress response prior to any visible mutant phenotypes. Accordingly, *csn* deletion strains are highly sensitive to oxidative stress conditions. We propose that the CSN acts at the interface between sensing of oxygen and / or reactive oxygen species (ROS) and induction of oxidative stress response.

Vo-3

Development and analysis of recombinant calcium sensors for use in live cell imaging of filamentous fungi

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Fluorescence techniques are extremely powerful tools for probing the structure and function of biomolecules. In particular, the use of fluorescence resonance energy transfer (FRET) is becoming increasingly important, providing information on interactions at the nanoscale. A variety of cameleon FRET indicators have been shown to respond to changes in calcium concentration *in vitro* and expressed in plant and mammalian cells; in filamentous fungi they have not yet been reported to work. We have designed calcium sensors based on cameleons using mCerulean as the donor and Venus as the acceptor fluorophore and expressed them in *Aspergillus niger*. Confocal analysis of these probes proved difficult due to the amount of bleedthrough from the donor into the acceptor emission channel. Therefore we chose Fluorescence Lifetime Imaging Microscopy (FLIM) as a new approach to FRET analysis. In the event of FRET, only the donor fluorescence lifetime changes whereas the lifetime of the acceptor remains the same as under no FRET conditions. Furthermore the fluorescence lifetime of a fluorophore is independent of the excitation intensity as well as the fluorophore concentration and not affected by moderate levels of photobleaching – significant advantages as compared to intensity based imaging.

FLIM analysis of mCerulean and a positive FRET control expressed in *A. niger* revealed a decrease of the donor fluorescence lifetime under FRET conditions, showing that we are able to positively identify FRET. In addition to the results obtained for the FRET controls we will present data acquired with different new calcium sensors varying in the linker between the mCerulean donor and Venus acceptor.

Overexpression of the Blr2 protein in *Trichoderma atroviride* results in higher photosensitivity.

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The influence of light on living organisms is critical, not only because of its importance as the main source of energy for the biosphere, but also due to its capacity to induce changes in the behaviour and morphology of nearly all forms of life. *Trichoderma atroviride* is a common soil fungus widely used as a biocontrol agent due to its capacity to parasitize phytopathogenic fungi of major agricultural importance. The application of *Trichoderma* in the field is based on the use of products of its asexual reproduction (conidia). Conidiation is tightly regulated by light and nutrient availability in this fungus. A pulse of blue/UVA light induces the synchronous production conidia at what had been the colony perimeter at the time of the pulse. In *Neurospora crassa* the proteins White Collar 1 and 2 (WC1 & WC2) regulate all responses to blue-light. WC1 is the photoreceptor, which together with WC2 transduces the light signal activating gene expression. Recently, we cloned two *T. atroviride* genes, named blue-light regulator one and two (*blr-1* and *blr-2*), homologues of the *N. crassa wc-1* and *wc-2*, respectively. The deduced protein sequence of BLR-1 indicates that it has all the characteristics of a blue-light receptor, whereas that of BLR-2 suggests that it could interact with BLR-1 through PAS domains to form a complex. Both BLR proteins were shown to be essential for photoconidiation in *Trichoderma*. The expression of both *blr* genes appears to be constitutive and the corresponding transcripts accumulate at low levels. We have obtained *T. atroviride* transformants that express *blr2* at high levels using the promoter of the *T. reesei pki* gene. The transformants require less light than the wild type to photoconidiate and produce more conidia per colony. Additionally, the expression of light regulated genes occurs earlier and reaches higher levels. Our data suggest that Blr2 is a limiting factor in light perception in *Trichoderma*.

Vo-5

Stages and impact of crosstalk between light response and cellulase signal transduction in *Hypocrea jecorina* (*Trichoderma reesei*)

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Light is one of the most important environmental cues for most living creatures, including humans. Collected light information is transferred to many different biological effectors, and proper perception of ambient light is essential for an organism to adapt to its environment. After the recent finding that cellulase signal transduction and light signalling are interconnected processes, we studied the light related phenomena in this fungus in more detail. By now we have evidence in hand, that indicates the involvement of the light regulator genes blr1, blr2 and env1, the H. jecorina orthologues of the N. crassa blue light regulators white collar-1, white collar 2 and the desensitization protein vvd, the subgroup III G-alpha protein Gna3 as well as a putative type a pheromone precursor in these connected pathways.

Moreover a subtraction hybridization study on genes rapidly upregulated after illumination further suggests the onset of unfolded protein response, ATP-synthesis and upregulation of proteins putatively involved in sugar uptake as well as ubiquitin-mediated protein degradation by the 26 S proteasome. The latter is further supported by the finding that an E3-ubiquitin ligase is regulated upon growth on cellulose in a light dependent way and binds to the cbh2 promotor. Having these data in hand we propose a model for the concerted regulation of cellulase gene expression in dependence of light.

The MAP kinase signaling network of *Cochliobolus heterostrophus*: modulation of gene expression and role in virulence and stress responses

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In filamentous fungi, conserved mitogen-activated protein kinase (MAPK) cascades are required for development, presumably because they convey environmental signals to alter gene expression. Pathogenicity MAP kinases, showing homology to yeast FUS3/KSS1, have been shown to be essential for virulence in several species, including *Cochliobolus heterostrophus*, a necrotrophic pathogen which is the agent of Southern corn leaf blight. We have characterized the phenotypes conferred by loss of function mutations in two MAPK genes, *BmHOG1* and *ChMPS1*, the phenotype of mutants in the third *C. heterostrophus* MAP kinase, *CHK1* was characterized previously. Mutants in *CHK1* and *ChMPS1* share a subset of common phenotypes including inability to produce conidia, autolytic appearance and dramatic reduction in virulence, even more pronounced in *ChMPS1* mutants. Formation of the small appressoria characteristic of this species was unimpaired in *ChMPS1* mutants. BmHOG1, on the other hand, is required for appressorium formation, but is dispensable for plant infection and conidiation. BmHOG1 is important for resistance to oxidative and hyperosmotic stresses. The normal virulence of *BmHOG1* mutants, despite their sensitivity to oxidative stress, implies that oxidative stress is not a major factor in the *C. heterostrophus* – maize interaction. It is likely, nevertheless, that resistance to oxidative stress provided an evolutionary advantage, and may be important for full fitness as a pathogen. We have identified genes whose expression reports the activity of each of the three pathways.

Vo-7

Linking genome-wide transcriptional changes to cellular physiology in oxidative stress-exposed *Aspergillus nidulans* cultures

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Genome-wide transcriptional changes triggered by diamide, H_2O_2 and menadione sodium bisulfite in *A. nidulans* vegetative tissues were recorded using double-printed DNA microarrays containing 3533 unique PCR-amplified probes (1). Evaluation of LOESS-normalized data indicated that 2499 gene probes were affected by at least one of the stress-generating agents and these effects were partly overlapping. The existence of gene groups solely responsive to changes in O_2^{2-}, $O_2^{\bullet-}$ concentrations or GSH/GSSG balance was demonstrated (1). In terms of cell physiology, membrane sterol glycosylation, protein *N*-myristoylation, MAP kinase pathways, cAMP-dependent kinase, COP9/signalosome and PAS-domain-containing kinase likely contributed to oxidative stress signalling and signal transduction. Stress response included altered cell cycle, cytoskeleton assembly, transcription, translation and up-regulation of antioxidative defence. Among primary metabolic pathways, citrate cycle and the biosynthesis of ergosterol were repressed with the concomitant induction of respiration. Biosynthetic pathways leading to the formation of metabolites with antioxidative properties and/or easily oxidized by reactive oxygen species, e.g. the amino acids Glu, Cys, Pro, the amino acid derivatives spermidine and γ-amino-butyrate, the carbohydrates fructose-1,6-bisphosphate and trehalose, the phospholipids phosphatidylethanolamine and cardiolipin as well as uric acid were induced. The production of the secondary metabolite sterigmatocystin was repressed up to the formation of averufin but was up-regulated in later steps.

(1) Pócsi, I., Miskei, M., Karányi, Z., Emri, T., Ayoubi, P., Pusztahelyi, T., Balla, G. and Prade, R.A. (2005) Comparison of gene expression signatures of diamide, H_2O_2 and menadione exposed *Aspergillus nidulans* cultures - linking genome-wide transcriptional changes to cellular physiology. BMC Genomics 6(182) http://www.biomedcentral.com/content/pdf/1471-2164-6-182.pdf