Saturday 31 March

Parallel session 1: Fungal Cell Biology

PS1.1

In Candida albicans the negative regulator of morphogenenis, Nrg1, is itself regulated at multiple post-transcriptional levels by kinase action

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The human fungal pathogen *C. albicans* is responsible for common mucosal disease with significant morbidity and among immunocompromised patients it can cause life-threatening hematogenously disseminated infections. A key virulence factor is its ability to switch from yeast to hyphal growth forms. This switch is positively regulated by a network of signal transduction pathways that target a panel of transcription factors that program the expression of hyphal-specific genes. Negative regulated is applied by the transcriptional repressor Tup1, targeted to the promoters of hyphal specific genes by the co-repressor Nrg1. While the signal transduction pathways targeting transcription factors have been extensively studied, less attention has been paid to the control of Nrg1 action. It has long supposed main mechanism of Nrg1 regulation is exerted through control of Nrg1 transcription which is repressed upon hyphal induction. However, Nrg1 transcript levels decline over a time scale of at least 100 minutes, whereas hyphal specific transcription can be detected much more rapidly than this. Here we show that upon hyphal induction, the repressive action of Nrg1 is relieved by a combination of alterations to protein stability, exclusion from the nucleus and alteration of binding to promoters of hyphal-specific genes. These changes are mediated by the action of a panel of kinases that target Nrg1.

PS1.2

Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae*

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Plant diseases are major causes of crop losses throughout the world and a significant constraint on worldwide agricultural production. Developing new means to control plant diseases is an important component of any strategy to ensure global food security. *Magnaporthe oryzae* is the causal agent of rice blast, one of the most devastating diseases of cultivated rice. Each year rice blast disease destroys enough rice to feed 60 million people. The availability of complete genome sequences for *M. oryzae* and its host rice, *Oryza sativa*, has provided the means to investigate this fungal-plant interaction in great detail. During plant infection *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor that is translated into mechanical force to cause rupture of the rice cuticle and entry into plant tissue. My research group is interested in determining the molecular basis of appressorium development and understanding the genetic regulation of the plant infection process by the rice blast fungus. Recently, we have shown that development of a functional appressorium is linked to cell cycle progression and programmed autophagic cell death of the fungal spore. New information on the role of a family of five septin GTPases in the function of appressoria will be presented, indicating that they serve an important role in translating turgor into mechanical force necessary for plant infection. The role of reactive oxygen species generation in the control of appressorium-mediated plant infection, will also be discussed.

PS1.3

Comparative Live-Cell Imaging Analyses of SPA-2, BUD-6 and BNI-1 in *Neurospora crassa* Reveal Novel Features of the Filamentous Fungal Polarisome

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A key multiprotein complex involved in regulating the actin cytoskeleton and secretory machinery required for polarized growth in fungi, is the polarisome. Recognized core constituents in budding yeast are Spa2, Pea2, Aip3/Bud6, and the key effector Bni1. Multicellular fungi display a more complex polarized morphogenesis than yeasts, suggesting that the filamentous fungal polarisome might fulfill additional functions. In this study we analyzed the subcellular organization and dynamics of SPA-2, BUD-6 and BNI-1 in a wide range of developmental stages of *Neurospora crassa*, in order to characterize the filamentous fungal polarisome more comprehensively, and identify potential differences to other fungal species. Our analyses showed that during early, unicellular developmental stages the filamentous fungal polarisome closely resembles the apical cap configuration known from yeasts, but during later, multicellular developmental stages all three polarisome components become spatiotemporally separated within the apical dome, and thus adopt a so far unknown configuration. Most notably, in vegetative hyphal tips BUD-6 accumulated as a subapical cloud excluded from the Spitzenkörper (Spk), whereas BNI-1 and SPA-2 partially colocalized with the Spk and the tip apex. Phenotypic analyses of gene deletion mutants revealed additional functions for BUD-6 and BNI-1 in septum formation, septal plug consolidation, tip re-polarization, cytokinesis, cell fusion regulation, and the maintenance of Spk integrity. Considered together, our findings reveal novel polarisome-dependent and -independent functions of BUD-6 and BNI-1, and their complex arrangement with SPA-2 in the apical dome of mature hypha represents a novel aspect of filamentous fungal polarisome architecture.

PS1.4

The *Aspergillus nidulans* Kinesin-3 Tail Is Necessary And Sufficient To Recognize Modified Microtubules

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Posttranslational microtubule modifications are numerous; however, the biochemical and cell biological roles of those modifications remain mostly an enigma. The *Aspergillus nidulans* kinesin-3 UncA uses preferably modified MTs as tracks for vesicle transportation. Here, we show that a positively charged region in the tail of UncA (amino acids 1316 to 1402) is necessary for the recognition of modified MTs. Chimeric proteins composed of the kinesin-1 motor domain and the UncA tail displayed the same specificity as UncA, suggesting that the UncA tail is sufficient to establish specificity. Interaction between the UncA tail and alpha-tubulin was shown using a yeast two-hybrid assay and in *A. nidulans* by bimolecular fluorescence complementation (BiFC). Our data show that specificity determination depends on the tail rather than the motor domain, as has been demonstrated for kinesin 1 in neuronal cells.

In a non-targeted Y2H approach interaction partners of this region were identified, because they are most likely involved in the recognition of MT subpopulations. Several candidates were confirmed using BiFC. Two are associated with vesicles; one is a predicted siderophore uptake transmembrane transporter and the other one was previously shown to be involved in ER to Golgi vesicle-mediated transport. The deletion of another fished interactor with similarity to Phosphatidylinositol 3- & 4-kinase family showed strongly reduced growth. Further characterization of a potentinal role in regulating the activity and specificity of UncA is in progress.

PS1.5

Active plus-end capture of dynein at astral microtubules increases the efficiency of anaphase B

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Dynein is involved in several cellular processes, including early endosomes motility. To do so, dynein concentrates at microtubule plus-ends from where it is released to mediate motility towards minus-ends. We recently have shown that in hyphae of *Ustilago maydis* an interaction between EB1 and dynactin capture ~25 dynein motors at apical microtubule plus-ends and that additional ~25 motors are stochastically clustering due to local crowding effects. In mitosis, Dynein concentrates at astral microtubule plus-ends, from where it is released to the cortex to exert force on the spindle, thereby powering rapid spindle elongation. Here, we investigate the dynein anchorage mechanism at these astral microtubule plus-ends in mitotic cells. In contrast to hyphal cells, only ~3 dynein motors concentrate at astral microtubule ends, and dynein is not anchored via EB1-dynactin. Instead, deletion of the plus-end binding protein Clip1 and the dynein activator Lis1 reduces the amount by one dynein each, respectively, which led to spindle position defects and 50% reduction in spindle elongation rate. Dynein numbers and spindle elongation rates were further reduced in a ΔClip1/Lis1↓ double mutant. However, in the absence of anchorage mechanisms ~1.7 dynein motors were still concentrated at astral microtubule plus-ends. Consequently, the spindle apparatus is still able to segregate chromosomes, albeit with much reduced efficiency. Mathematical modelling suggests that this can be a consequence of stochastic clustering effects at microtubule ends. These results suggest that mitosis can function without active anchorage. In order to increase the efficiency of chromosome segragation, Clip1 and Lis1-dependent processes support dynein concentration at astral microtubule ends.

PS1.6

Candida albicans: sensing the host environment

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Candida albicans, an opportunistic pathogen and a human commensal, is found almost exclusively in association with a host. Intestinal tract colonization by this organism is common in humans and disease is thought to arise due to overgrowth or escape of organisms from the gut. In the host, the organism's ability to sense cues from the environment would be expected to enhance its growth and survival. C. albicans responds to numerous environmental cues that could be encountered in the host such as temperature, pH, presence of O2, CO2, nutrients or antimicrobial compounds, and stress conditions. Our studies focus on the ability of C. albicans to sense contact with a surface and to sense the environment within the intestinal tract. These types of sensing mechanisms promote the organism's ability to sense its location within the host, the nature of the tissue that it is encountering and the status of the host's immune response. As a result of these sensing mechanisms, the organism controls its physiology so that it maintains benign colonization in a healthy host but becomes a destructive pathogen that invades host tissue in a compromised host.

PS1.7

A steep phosphoinositide phosphate gradient is critical for filamentous growth in *Candida albicans*

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Membrane phospholipids, such as phosphoinositide phosphates, despite being minor membrane components, have been shown to be required for cytoskeleton organization, G-protein signaling, cell polarity and morphogenesis in a range of organisms. In *Candida albicans*, neither PI(3,4,5)P_3 nor PI-3-kinase homologs have been found, raising the possibility that the PI(4,5)P_2 fulfills some functions of PIP_3. In this organism there is a single PI(4)P-5-kinase (encoded by *MSS4*) and three PI-4-kinases (encoded by *LSB6*, *STT4* and *PIK1*). In the yeast *S. cerevisiae*, both Mss4 and Stt4 are required for viability, organization of the actin cytoskeleton and are localized to the plasma membrane.

We examined whether PI(4,5)P_2 is required for *C. albicans* filamentous growth. We have generated strains in which the level of the Stt4 or Mss4 PI-kinases can be manipulated using the Tetracycline repressible promoter system. In repressive conditions, the *stt4* and *mss4* mutants are viable, yet defective in filamentous growth. Using a fluorescent lipid associated reporter, we have observed a striking PI(4,5)P_2 asymmetry in budding cells and a steep gradient which occurs concomitant with germ tube emergence. Both sufficient PI(4)P synthesis and an intact actin cytoskeleton are necessary for this steep PI(4,5)P_2 gradient. In contrast, neither microtubules nor asymmetrically localized mRNAs are critical for this gradient. Furthermore, the Mss4 protein is localized to the tip of the bud and hyphal filament. Our results indicate that a gradient of PI(4,5)P_2, generated in part by filament tip-localized Mss4 and the slow diffusion of plasma membrane PI(4,5)P_2, is crucial for the yeast to filamentous growth transition.

PS1.8

In Vivo Nonlinear Spectral Imaging Of Fungi

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Non-linear microscopy combined with fluorescence spectroscopy is known as non-linear spectral imaging (NLSI), providing simultaneously the specimen morphology and (auto)fluorescence spectra. Hence, it allows deducing the biochemical composition, while distinguishing different parts of the tissue.

We introduce NLSI to in vivo monitor the metabolism of fungi. Fungi are consumables (food) and are utilized to produce industrial and pharmaceutical compounds, requiring quality control. With NLSI, we present a fast method to determine the metabolic state and relate it to protein production. Moreover, we introduce NLSI and NLSI-data processing tools as an easy to use method, capable of addressing a broad range of microbiological questions.