Workshop VI

Fungal Cell Biology
Chair: Nick Read

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ANALYSING THE CELL BIOLOGY OF LIVING FUNGAL CELLS

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In recent years there have been important developments in live-cell analytical techniques (e.g. confocal microscopy, vital and GFP probes, and laser tweezer micromanipulation) applied to studies on filamentous fungi. Much of the work of my lab has focused on developing and optimising these powerful technologies in applications to provide novel insights into the biology and dynamics of living fungal cells. We are currently using these approaches to investigate different aspects of the cell biology of macroconidial germination and early colony development in *Neurospora*. The process of hyphal homing and fusion (anastomosis) between conidial germlings of labelled has been imaged using different vital dyes and GFP probes. Both germ tubes and another type of specialised, morphologically distinct hypha (called a conidial anastomosis tube [CAT]) are produced by macroconidia. In wild type strains, CATs are thinner than germ tubes and do not undergo branching. In contrast to germ tubes, conidial anastomosis tubes grow towards each other. We have developed a simple laser tweezer technique to optically manipulate whole spores and germlings. When homing germlings are moved relative to each other the CATs subsequently reorientate themselves and grow back towards each other. This provides clear evidence for the existence of, as yet unknown, diffusible chemotropic signals being involved in the homing response of CATs. This experimental manipulation of macroconidia is being used in assays to determine whether strains of different genetic backgrounds can home towards or fuse with each other. In this way we have shown that fusion between CATs is independent of mating type. Nuclear movement, and continuity of the microtubular cytoskeleton between fused germlings, have been imaged. Endocytosis, as indicated by the internalisation of the membrane-selective probe FM4-64, is initiated after spore hydration before the emergence of germ tubes or CATs. Interestingly, the Spitzenkörper in germ tubes is not stained by the dye as it is in vegetative hyphae suggesting differences in the pathways of vesicle trafficking in these different cell types.

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THREE DIMENSIONAL IMAGE ANALYSIS OF PLUGGING OF THE SEPTAL PORE BY WORONIN BODY IN A. ORYZAE DURING HYPHAL LYSIS INDUCED BY HYPOTONIC SHOCK

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Woronin body, an organelle found in some groups of filamentous fungi is known to function in plugging the septal pores after hyphal damage. Its elucidation had been limited exclusively to electron and light microscopic observations until the molecular characterization of Neurospora crassa hex-1 involved in Woronin body formation (Jedd and Chua, 2000). Disruption of Aspergillus oryzae hexA gene homologous to N. crassa hex-1 caused disappearance of Woronin body. In the study to examine conditions inducing hyphal lysis, we established the time-lapse observation method upon hypotonic shock during which many hyphal tip regions grown on agar media burst out their cytoplasmic constituents after adding water. This provided the evidence that the hexA disruptant conferred the defect to prevent extensive loss of cytoplasm at the second compartment. Moreover, fluorescent dots of EGFP-HexA fusion proteins localized at the center of septa adjacent to lysed apical compartments while very few of them were detected at the center of septa under normal culture conditions, suggesting the plugging of Woronin body at the septal pore upon hypotonic shock. In order to more spatially visualize this phenomena, we utilized confocal microscopy and 3D reconstruction, and successfully visualized the septal pore as a dark region surrounded by green fluorescence of EGFP-fused secretory protein, RNase T1, on the septum. Dual staining with DsRed2-HexA revealed the red fluorescent Woronin bodies at the septal pore adjacent to lysed apical compartments.

CELL WALL METABOLISM OF ASPERGILLUS NIDULANS

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The value of filamentous fungi as production organisms for a whole range of very diverse products is undisputed. However, compared to unicellular organisms, their mycelial morphology poses restrictions and challenges to their industrial usage. The complex interplay between different morphologies and productivity still lies mainly in the dark, due to the lack of understanding of morphogenesis on a very fundamental level.
The fungal cell wall, its biosynthesis and the regulatory mechanisms governing its composition, certainly play an important role in establishing and maintaining a morphology.
Therefore three genes involved in the formation of precursors needed for cell wall synthesis have been cloned and characterised, and their expression, and three more genes from the same metabolic pathways, under different environmental conditions has been analysed.

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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IN SEARCH OF A LINK BETWEEN MORPHOGENESIS AND NUCLEAR DIVISION IN THE FILAMENTOUS FUNGUS ASHBYA GOSSYPII

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How mitosis is spatially regulated in a multinucleated filamentous fungus is largely unknown. We study this problem in the ascomycete Ashbya gossypii, which is closely related to S. cerevisiae on a genome scale. Yet, A. gossypii displays an entirely different pattern of growth, leading to branched filaments whose nuclei multiply by asynchronous mitosis. This further raises the question whether, unlike in budding yeast, hyphal morphogenesis is independent of cell cycle stage in A. gossypii.
In S. cerevisiae, the septins are thought to link bud formation to mitosis by recruiting Hsl1p and Hsl7p, which are required for Swe1p inactivation and entry into mitosis. The proper assembly and organization of the septin collar at the neck is essential for the function of these cell cycle regulators. In A. gossypii, homologues to all key players of this morphogenesis checkpoint exist. To begin to investigate possible connections between the cell cycle and morphogenesis we have focused on analyzing the structure and function of septin proteins in A. gossypii by using a Sep7-GFP strain. We detected some septin structures that were never described in yeast so far. Interestingly, most of the structures in A. gossypii seem to be composed of parallel bars, an instance which in yeast is only seen in certain mutants and is associated with higher Swe1p kinase activity. Heterologous expression of AgSep7-GFP in S. cerevisiae leads to the formation of normal yeast structures with continuous rings and double rings. This implies that the observed structures are not inherent to the Sep7 protein, but rather the result of different regulation or interaction in the two organisms.
To study whether the absence of septin structures would influence mitosis, we evaluated nuclear density in cdc3, cdc10, and cdc12 deletion mutants. All strains were viable and showed altered morphology, reduced radial growth rates and failed to sporulate and disrupted the septin structures. Yet, neither any of the described septin deletions nor an hsl1 deletion had an obvious effect on nuclear density. Possibly, A. gossypii has additional mechanisms to control Swe1p activity or to compensate the lack of Swe1p inhibition, or Swe1p is regulated independently of the septin pathway. Arguing against this last hypothesis is the fact that Hsl7-GFP localizes into ring like structures that highly resemble those observed with Sep7-GFP.
Current research focuses on analyzing time lapse movies of cells carrying both GFP labeled nuclei and Sep7-GFP to examine whether there is a correlation between changes in the septin structures and nuclear division.

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A NEW MODEL OF FUNGAL MITOTIC REGULATION.

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Mitosis in Aspergillus nidulans is closed and takes place within nuclei with an intact nuclear envelope. This necessitates transport of mitotic regulators and tubulin to the nucleus to trigger mitosis and subsequent removal of such proteins from nuclei to allow exit from mitosis. The nuclear pore complex (NPC) is a huge structure many times the size of a ribosome and during interphase all nuclear import and export occurs through the transport channel of the NPC. In A. nidulans, mitosis is regulated by the essential NIMA mitotic-specific protein kinase. Two genes isolated as extragenic suppressors of nimA1 encode conserved interacting proteins that reside at the NPC. Proteins residing at the NPC are termed nucleoporins. The NIMA interacting nucleoporins, SONA and SONB, may therefore play a role in the regulation of mitosis. Supporting this hypothesis, both SONA and SONB proteins are dramatically released from the NPC after activation of NIMA during prophase and return to the NPC at telophase as NIMA is degraded. When SONA and SONB are removed from the NPC during mitosis, nuclear reporter constructs leak from nuclei and soluble tubulin enters nuclei. NIMA is able to markedly modify the properties of the NPC because ectopic expression of NIMA during S-phase arrest promotes dissociation of SONB from the NPC, release of nuclear reporters from the nucleus, and nuclear entry of cytoplasmic tubulin. To see which nucleoporins are removed from the NPC during mitosis and which remain, other nucleoporins have been GFP-tagged utilizing the genome sequence of A. nidulans and gene replacements. Some, but not all, nucleoporins are lost from the NPC during mitosis. Therefore, the protein makeup of the NPC is clearly modified during mitosis as are its transport properties. We propose that NIMA regulates the properties of the NPC during mitosis by promoting release of selective nucleoporins from the NPC which opens the transport channel of the NPC from prophase to anaphase. Depending on the relative binding properties of proteins they will either leak from nuclei or concentrate within nuclei during mitosis. During telophase, NIMA is degraded to allow return of nucleoporins to the NPC and reestablishment of regulated transport to promote cells to exit mitosis and enter G1. Our data present a new model of mitotic regulation and provide a mechanism by which NIMA regulates entry and exit from mitosis.