

IV
MORPHOLOGY AND
REPRODUCTION

Chair:

Stephanie Pöggeler

& Susanne Zeilinger



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The application of targeted functional genomics to study the mitotic regulation of the nuclear pore complex in *Aspergillus nidulans*

Stephen A Osmani^{1*}, Jonathan Davies¹, Eduardo A Espeso², Javier Fernández Martínez², Hui-Lin Liu¹, Aysha H. Osmani¹

¹ *The Ohio State University*

² *Centro de Investigaciones Biológicas (C.S.I.C.)*

Previous studies have demonstrated that five nuclear pore complex proteins (nucleoporins) are dispersed from the nuclear pore complex (NPC) specifically during *A. nidulans* mitosis whereas three nucleoporins remain at a minimal core structure. To further understand the magnitude and regulation of the mitotic modification of the *A. nidulans* NPC we have identified 30 nucleoporin proteins. All encoding genes have been deleted and most endogenously GFP and/or mRFP tagged.

To identify essential genes, we used heterokaryon rescue. This analysis identified 16 of the 30 genes to be essential. Of the 14 non-essential genes, deletion of 5 causes marked temperature sensitivity, 2 deletions cause cold sensitivity and 1 causes sterility. Analysis of the growth phenotypes and nuclear morphology indicates that deletion of different nucleoporin genes leads to a wide variety of nuclear defects including many apparent mitotic defects. This suggests there is not a mechanism by which the composition of the NPC can be monitored to arrest cell cycle progression when NPC proteins are limiting.

Live cell imaging of the nucleoporins that constitute the *A. nidulans* NPC indicates at least 11 specifically disperse from the NPC during mitosis whilst at least 11 remain at a minimal core NPC structure. In addition, one nucleoporin relocates from the NPC to the mitotic chromatin during mitosis and then back to the NPC during exit from mitosis. The dynamics of the dispersed nucleoporins indicate that the dispersal of these proteins occurs rapidly during prophase and that there is a stepwise reassembly of a functional NPC during the transition from metaphase through to G1. The study also demonstrates that the nuclear envelope undergoes membrane fission at not one, but two distinct locations to generate two nuclei from one.

This work demonstrates the power of targeted genomics when studying large protein complexes with essential functions that are regulated in a complex manner. The technologies applied should be applicable to any set of interacting proteins of interest and lay the foundation for moving from targeted approaches to true genome wide studies in *A. nidulans*.

Endocytosis in *Neurospora crassa*

Eric R Kalkman^{1*}, Mari Valkonen², Nick D Read¹

¹ Fungal Cell Biology Group Institute of Cell Biology King's Buildings University of Edinburgh Edinburgh EH9 3JH United Kingdom

² VTT Biotechnology Tietotie 2 PO Box 1500 Espoo FIN-02044 VTT Finland

Endocytosis has been well characterized in budding yeast and animal cells, and to a lesser extent in plant cells. In contrast, much less is known about this process in filamentous fungi although it is likely to have important roles in membrane recycling, membrane degradation, and the uptake of signal molecules. This presentation will provide evidence from *Neurospora crassa* which supports the occurrence of endocytosis in filamentous fungi. This evidence includes: 1) membrane-selective markers of endocytosis in filamentous fungi (FM 1-43 and FM 4-64) are internalized by spores and hyphae; 2) markers of fluid-phase endocytosis (Lucifer Yellow and FITC-dextran) are internalized by protoplasts; 3) internalization of endocytosis markers is active and not by diffusion because it is reversibly inhibited by azide or cold treatment; 4) internalization of FM 4-64 is actin-mediated because it is inhibited by Latrunculin B and by Cytochalasin D; and 5) the genomes of several filamentous fungi encode a complex endocytic protein machinery. In addition, we show behaviour and localization of two endocytic proteins labelled with GFP, as well as a GFP labelled protein that is internalized by endocytosis.

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AbpA: a tool to study endocytosis in *Aspergillus nidulans*

Lidia Araujo-Bazan^{*}, Eduardo A Espeso, Miguel A Peñalva

Centro de Investigaciones Biológicas, C.S.I.C., Ramiro de Maeztu, 9. 28040 Madrid (Spain)

Endocytosis is important for many plasma-associated functions such as cell growth regulation, cell polarity establishment or cell motility, because of its role in the control of protein and lipid composition of the plasma membrane, control of cell surface area, regulation of signal pathways and uptake of nutrients. Although endocytosis has been extensively studied, very little is known in the model organism *Aspergillus nidulans*. The morphological and distinctive way of growth (extremely polarised) of *Aspergillus*, contrasting with those from the organisms frequently used for this kind of studies like *Saccharomyces cerevisiae*, are differences exploited in this study. In filamentous fungi endocytosis not only plays an important role on recycling the cell plasma membrane but also on transducing the information from the ambient as the apical cell constantly grows invading new environments. One third of the endocytic proteins directly regulate actin assembly and/or bind to actin. The actin-binding-protein 1 (Abp1p) is a highly conserved actin-binding protein linking functions of the actin cytoskeleton to endocytosis from yeast to mammals. Because of this, we chose the *Aspergillus nidulans* Abp1p orthologue to study the endocytotic process. The unique *ABP1* orthologue in *Aspergillus* was found using bioinformatics, AN8873.2, being designated as *abpA*. After cDNA sequencing, we determined that AbpA shows strong similarity with Abp1p having one conserved cofilin domain and two, instead of one, SH3 domains. We have generated green and monomeric red fluorescent tagged versions of AbpA to analyse its *in vivo* subcellular localization. AbpA, as well as yeast Abp1p, localizes to cortical patches. Similar subcellular localisation has been determined for actin and the amphiphysin orthologue AnRvs167. AbpA cortical patches, as expected, are mobile and their lifetime has been determined. Using latrunculin B we have observed that AbpA patches movement is actin filament dependent. The distribution of AbpA in *Aspergillus* is of interest. There is an accumulation of patches at the apex but also can be observed throughout the cell, which suggests that endocytosis is not a process taking place exclusively at the cell tip. These results clearly show that AbpA can be used as a tool to study the localisation and dynamics of the endocytosis process in *Aspergillus*. In addition, the marked apex localisation of AbpA makes it as a suitable probe for studying polarised growth in this filamentous fungus.

Pheromones and pheromone receptors are required for proper sexual development in homothallic ascomycetes

Severine Mayrhofer¹, Jan M Weber², Stefanie Pöggeler^{2*}

¹ *Stratagene, Gebouw California, Hogehilweg 15, 1101 CB Amsterdam Zuidoost The Netherlands*

² *Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität, 44780 Bochum, Germany*

The homothallic, filamentous ascomycete *Sordaria macrospora* is self-fertile and produces sexual fruiting bodies (perithecia) without a mating partner. Even so *S. macrospora* transcriptionally expresses two pheromone-precursor genes (ppg1 and ppg2) and two pheromone-receptor genes (pre1 and pre2). The proteins encoded by these genes are similar to a-factor-like and a-factor-like pheromones and to G-protein-coupled pheromone receptors of the yeast *Saccharomyces cerevisiae*. It has been suggested that in *S. macrospora*, PPG1/PRE2 and PPG2/PRE1 form two cognate pheromone/receptor-pairs.

To investigate whether the a-factor-like pheromone/receptor-pair is functional in *S. macrospora* a heterologous yeast assay was used. Our results show that the *S. macrospora* a-factor-like pheromone precursor PPG1 is processed into an active pheromone by yeast MATa cells. The *S. macrospora* PRE2 receptor was demonstrated to be a G-protein-coupled receptor. In yeast MATa cells lacking their endogenous Ste2p receptor, the *S. macrospora* PRE2 receptor is able to complement the defect and facilitates all aspects of the pheromone response (Mayrhofer and Pöggeler 2005).

In order to investigate the functions of the pheromone/receptor system in the homothallic *S. macrospora*, we deleted (D) pheromone-precursor genes (Dppg1, Dppg2) and receptor genes (Dpre1, Dpre2) and generated single- as well as double-knockout strains. No effect on vegetative growth, fruiting-body and ascospore development was seen in the single pheromone-mutant and receptor-mutant strains, respectively. However, double-knockout strains lacking any compatible pheromone/receptor-pair (Dpre2/Dppg2, Dpre1/Dppg1) and the double-pheromone mutant (Dppg1/Dppg2) displayed a drastically reduced number of perithecia and sexual spores, whereas deletion of both receptor genes (Dpre1/Dpre2) completely eliminated fruiting-body and ascospore formation. The results suggest that pheromones and pheromone receptors are required for optimal sexual reproduction of the homothallic *S. macrospora* (Mayrhofer *et al.* 2006).

- Mayrhofer S, Pöggeler S (2005) Functional characterization of an a-factor-like *Sordaria macrospora* peptide pheromone and analysis of its interaction with its cognate receptor in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 4: 661-672
- Mayrhofer S, Weber JM, Pöggeler S (2006) Pheromones and pheromone receptors are required for proper sexual development in the homothallic ascomycete *Sordaria macrospora*. *Genetics* in press

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A serum response factor homologue interacts with a mating-type protein and is required for fruiting body development in *Sordaria macrospora*

Nicole Nolting* , Stefanie Pöggeler

Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany

MADS-box transcription factors control diverse developmental processes in land plants, metazoans and fungi. To analyze the involvement of MADS-box proteins in fruiting-body development of filamentous ascomycetes, we isolated the *mcm1* gene from the homothallic ascomycete *Sordaria macrospora*, encoding a putative MCM1 homologue. A phylogenetic analysis, including MADS-box genes of fungal, plant and metazoan origin, revealed that the *S. macrospora mcm1* gene is a member of the metazoan SRF-gene family. Deletion of the *S. macrospora mcm1* gene displayed that it is not only involved in vegetative growth but also in sexual reproduction. The *S. macrospora* $\Delta mcm1$ deletion strain shows a hyperbranching phenotype during vegetative growth and is unable to produce perithecia or ascospores during sexual development. A yeast two hybrid- and in-vitro analyses demonstrated that the *S. macrospora* MCM1 protein has the capability to interact with the mating-type protein SMTA-1. These results suggest an involvement of the *S. macrospora* MCM1 protein in the transcriptional regulation of mating-type specific genes and fruiting-body development.

A yeast two-hybrid screen identifies the NADPH-oxidase complex as a potential target for RacA regulation

Mark Arentshorst*, Cees AMJJ van den Hondel, Arthur FJ Ram

Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

RacA encodes a small GTPase that belongs to the sub-family of Rho-like GTPases. Rac proteins are highly conserved among eukaryotic cells, but are absent in yeasts. One of the important functions of RacA, both in filamentous fungi and in other eukaryotic cells, is that it is involved in controlling and organizing actin polymerization and thereby controlling cellular morphology. In order to identify proteins that interact with the activated form of RacA (RacA^{G12V}), a yeast two hybrid screen was performed using the RacA^{G12V} protein as a bait. Gateway-compatible yeast two hybrid cDNA libraries from a variety of growth conditions were constructed. The two-hybrid screen resulted in 15 positive transformants. Sequencing the inserts revealed the isolation of two Rac interacting proteins. The first insert encodes a hypothetical protein without known function. The second insert is highly homologous to the mammalian p67^{phox} protein. p67^{phox} is part of the NADPH oxidase complex which is active in phagocytes during phagocytosis of invading microbes. The NADPH oxidase complex produces superoxide (O₂⁻) a precursor of microbicidal reactive oxygen species (ROS), such as H₂O₂. In mammalian cells it is well described that the activation of the NADPH oxidase complex is regulated by Rac. Our results suggest that RacA from *A. niger* has, in addition to its role in fungal morphology, also has a additional function in regulating NADPH oxidase activity.

In *A. nidulans*, it has been shown that reactive oxygen species generated by the NADPH-oxidase complex activity regulate sexual development without affecting the asexual conidiation cycle (Lara-Ortiz *et al.*, 2003). Since *A. niger* lacks the sexual developmental cycle, there might be an additional function for RacA in *A. niger*. Yeast transformants containing the p67^{phox} homolog as a RAC^{G12V}-interacting protein were predominantly obtained from the (asexual) conidiation library. This suggests that the NADPH-complex might play a role during asexual development in *A. niger*. The observation that the *racA* deletion strain has a conidiation defect is in line with this hypothesis. A possible role of the RacA regulated NADPH-oxidase complex in *A. niger* on conidiation will be studied by constructing the p67^{phox} disruption mutant.

References:

Lara-Ortiz T, Riveros-Rosas H, Aguirre J. *Mol Microbiol.* 5, 1241-1255. 2003.