PS5.1

Functional analysis of ERMES and TOB (SAM) complex components in *Neurospora crassa*.

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The *Neurospora crassa* TOB complex (topogenesis of beta-barrel proteins), also known as the SAM complex (sorting and assembly machinery), inserts a subset of mitochondrial outer membrane proteins into the membrane—including all beta-barrel proteins. The TOB core complex contains Tob55, Tob38, and Tob37. Each of these proteins is essential for viability of *N. crassa*. The TOB holo complex contains an additional protein called Mdm10. Deficiency of any of these proteins results in impaired assembly of beta-barrel proteins. Lack of Mdm10 also results in a phenotype of enlarged mitochondria. The Mdm10 protein has also been shown to be a member of the ERMES complex (endoplasmic reticulum-mitochondria encounter structure) in *Saccharomyces cerevisiae*. The complex is thought to function in lipid and calcium exchange between the two organelles. Other members of the ERMES include the Mdm12, Mmm1, and Gem1 proteins. We have shown that deficiency of Mdm12 or Mmm1 also results in the presence of enlarged mitochondria and impaired beta-barrel protein assembly into the mitochondrial outer membrane while lack of Gem1 does not. The possibility that different domains of the proteins of the TOB and ERMES complexes are responsible for specific interactions and functions is currently being explored.

PS5.2

On mitochondrial genes, genomes and proteomes

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A key event in eukaryotic evolution, the symbiotic introduction of mitochondria (mt), occurred a billion or more years ago. This symbiosis contributed roughly $1/10^{th}$ the genetic material to extant eukaryotes, which apparently all have or once had a mitochondrion. Most genes of mt origin are no longer encoded in the mitochondrial but in the nuclear genome. Therefore massive protein targeting has to occur, as well as a structural organization specific to mitochondria, although an overall conservation of bacterial features can be expected. Questions that we have been interested in are (i) identification of the complete mitochondrial proteome in yeast and other fungal species, (ii) organization of mitochondrial function in higher-order structures (complexes and super-complexes; in comparison to *E. coli*), and (iii) analysis of the ribonucleo-protein complex RNase P, which in some instances has an mtDNA-encoded catalytic RNA subunit, whereas in others the RNA is imported from the cytoplasm.

We have further followed up on the evolutionary question of mitochondrial origins using both mt genome data and nuclear genes of mt origin - by rooting of the eukaryotic tree and identification of the closest extant bacterial relatives of mitochondria. Our results support the view that eukaryotes are ancient, that the mitochondrial endosymbiosis is a relatively recent event, and that the root of the eukaryotic tree is between the monophyletic 'unikonts' and 'bikonts'. Whether or not *Rickettsia*-like bacteria are the sistergroup of mitochondria as commonly assumed remains an open question.

PS5.3

Mitochondrial dynamics and organismal ageing in *Saccharomyces cerevisiae*

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Mitochondria are essential organelles of energy conversion and other vital pathways in most eukaryotic organisms. The dynamic behaviour of mitochondria, which includes movements of the organelles within the cell as well as opposing fusion and fission processes, is tightly controlled by a set of proteins. Among these, large Dynamin-related GTPases and several proteases in addition to other factors play key roles in this control. Previously, it was shown that reduced mitochondrial fission leads to a network-like morphology, decreased sensitivity for the induction of apoptosis and a remarkable extension of both replicative and chronological lifespan in one of the most important model systems for biological ageing, *Saccharomyces cerevisiae*. On the other hand, promoting mitochondrial fission by deleting the fusion gene *Mgm1* leads to a striking reduction of both replicative and chronological lifespan with a substantial increase of sensitivity to apoptosis elicitation via the reactive oxygen species hydrogen peroxide. It has been shown that Mgm1p is proteolytically cleaved by the rhomboid protease Pcp1p to yield a large and a small isoform of Mgm1p which are both required for efficient fusion of mitochondria. To investigate the importance of this process on mitochondrial functionality and ageing we analysed *Pcp1* deletion mutants containing respiratory competent (rho^+) and respiratory incompetent (rho^0) mitochondria. Senescent Δ*pcp1* rho^+ but not senescent Δ*pcp1* rho^0 cells display a striking mitochondrial morphotype reminiscent of stress-induced mitochondrial hyperfusion (SIMH) in mammalian cells. This correlates with a robust increase of replicative and chronological lifespan of Δ*pcp1* rho^+ cells compared to the Δ*pcp1* rho^0 cells. These findings suggest that the SIMH-like process in Δ*pcp1* rho^+ positively influence ageing by prolonging mitochondrial functionality. Our results bear important clues for translational research to intervene into age-related degenerative processes in multicellular organisms including humans.

PS5.4
Mitochondrial dynamics in yeast
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Mitochondria are amazingly dynamic organelles. They continuously move along cytoskeletal tracks and frequently fuse and divide. These processes are important for maintenance of mitochondrial functions, for inheritance of the organelles upon cell division, for cellular differentiation, and for apoptosis. As the machinery of mitochondrial biogenesis and inheritance has been highly conserved during evolution, it can be studied in simple model organisms such as yeast. By systematic screening of comprehensive yeast mutant collections and functional analyses we have identified novel components and cellular pathways required for mitochondrial fusion, division, motility, and maintenance of respiratory activity. These data provide a comprehensive picture of the molecular processes required for mitochondrial biogenesis in a simple eukaryotic cell. Our recent studies focus on the roles of mitochondrial cell cortex attachment and myosin-dependent movements in mitochondrial inheritance.

PS5.5
Mitochondrial Protein Quality Control Influences Lifespan and Stress Adaptation in *Podospora anserina*
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Mitochondria are essential organelles of eukaryotic organisms. Maintaining their integrity is of key relevance, as mitochondrial dysfunction has been linked to a number of adverse phenomena such as aging or the development of degenerative diseases, e.g., Parkinson's and Alzheimer's disease. A complex network of different quality control (QC) pathways has evolved to meet this necessity. Protein QC in mitochondria is accomplished by several proteases located in the intermembrane space, the inner membrane (IM) and the mitochondrial matrix (MM).

Here, we present recent findings regarding the biological role of the *i*-AAA protease, located in the IM, and the ClpXP complex, located in the MM, in the fungal aging model *Podospora anserina*. Deletion of the gene *PaIap*, coding for the *i*-AAA protease, leads to mutant strains that display a pronounced increase in their healthy lifespan under standard growth conditions but are sensitive to heat stress. Consistently, growth at elevated temperatures leads to higher PaIAP abundance in wild type strains. These observations suggest that *P. anserina*'s *i*-AAA protease is part of an inducible QC system that has evolved to allow survival under fluctuating environmental conditions.

The phenotype of *PaClpP* deletion strains strongly resembles that of the *PaIap* deletion strains. Significantly, it was possible to complement the Δ*PaClpP* mutants by heterologous over-expression of the human *ClpP*-cDNA in the fungal deletion background. Although further experiments are required, it now seems feasible to use *P. anserina* for the functional characterization of the as of yet poorly studied eukaryotic Clp proteases.

PS5.6

A Mitochondrial Molecular Marker for estimating Arbuscular Mycorrhizal Fungal Biomass in Soil and Roots

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Arbuscular Mycorrhyzal Fungi (AMF) are significant contributors to the growth and health of a majority of plant species worldwide. AMF enhance nutrient assimilation from the soil (phosphorous, nitrogen) and enhance plant resistance to drought and pathogen infections. Several qualitative, quantitative and semi-quantitative tools are currently in use to estimate biomass and species diversity of AMF associated with soil and roots. Among them, the use of real-time PCR techniques has been validated in several systems, *in vitro*, in the greenhouse and in field samples, with various degrees of success. The recent sequencing of the first mitochondrial genome of an AMF species, *Glomus intraradices,* has provided a new toolbox to be used among others, for AMF identification and quantification purposes. Herein we present the mitochondrial gene *nad1* as a candidate molecular marker for the estimation of AMF biomass in the soil and roots. We analysed the sequence polymorphisms associated with *nad1* in a panel of reference *Glomus* species. We used a *Glomus*-specific region within the *nad1* gene to design a TaqMan tool for the quantification of several species of *Glomus* in environmental soil samples. We present data on the validation of the tool *in vitro*, in the greenhouse and in agricultural soil and root samples against a diverse collection of *Glomus* species.

PS5.7

The mitochondrial genome of the wood-decaying basidiomycete *Phlebia radiata* is the largest in size (156 kb) among fungi and contains a 6 kb inversion, stretches with repetitive elements and long introns invaded with homing endonucleases

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The mitochondrial (mt) genome of the wood-decaying, white rot saprobic basidiomycete *Phlebia radiata* is the largest in fungi, 156 kb in size, sequenced so far. The mt genome of *P. radiata* is a single circular dsDNA molecule with average GC content of 31.1 %, and reveals several novel features such as a 6 kb duplication-inversion region containing several parallel, anticlockwise orientated open reading frames (ORFs). The exceptionally large size of the mt genome is explained by frequent splicing of the conserved genes with long introns (0.5-3 kb in size), the presence of additional unknown ORFs, existence of the large duplication-inversion region, and most of all, due to long stretches carrying repetitive sequence elements with variant motifs. A few of the repetitive elements containing regions indicate transposable, plasmid or viral origin. The mt genome contains the 14 conserved genes coding for essential proteins participating electron transfer and oxidative phosphorylation, the SSU and LSU rRNA genes, a gene for ribosomal protein subunit 3, and 28 tRNA genes, of which 11 are anticlockwise orientated. Phylogeny of the 14 protein coding sequences confirms current fungal taxonomy. Over 50 homing endonucleases of LAGLIDADGD and GYI-YIG types are recognized within the long introns splicing most of the 14 essential mt protein coding genes, except for *atp6* and *nad2* genes. The repetitive stretches, duplication-inversion and high amount of intron-homing endonucleases are features pointing to exceptional genetic flexibility and allowance of DNA recombination, which have not been recognized to this extent – at genome level – in fungal mitochondria previously.

PS5.8

Phylogenetic analysis of the complete mitochondrial genome of *Madurella mycetomatis* confirms its taxonomic position within the order Sordariales

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Background: *Madurella mycetomatis* is the most common cause of human eumycetoma. The genus *Madurella* has been characterized by overall sterility on mycological media. Due to this sterility and the absence of other reliable morphological and ultrastructural characters, the taxonomic classification of *Madurella* has long been a challenge. Mitochondria are of monophyletic origin and mitochondrial genomes have been proven to be useful in phylogenetic analyses.

Results: the first complete mitochondrial DNA genome of a mycetoma-causative agent was sequenced using 454 sequencing. The mitochondrial genome of *M. mycetomatis* is a circular DNA molecule with a size of 45,590 bp, encoding for the small and the large subunit rRNAs, 27 tRNAs, 11 genes encoding subunits of respiratory chain complexes, 2 ATP synthase subunits, 5 hypothetical proteins, 6 intronic proteins including the ribosomal protein *rps*3. In phylogenetic analyses using amino acid sequences of the proteins involved in respiratory chain complexes and the 2 ATP synthases it appeared that *M. mycetomatis* clustered together with members of the order Sordariales and that it was most closely related to *Chaetomium thermophilum.* Analyses of the gene order showed that within the order Sordariales a similar gene order is found. Furthermore also the tRNA order seemed mostly conserved.

Conclusion: Phylogenetic analyses of fungal mitochondrial genomes confirmed that *M. mycetomatis* belongs to the order of Sordariales and that it was most closely related to *Chaetomium thermophilum*, with which it also shared both a comparable gene and tRNA order.

Sunday 1 April

Parallel session 6: ROS, Autophagy and Apoptosis

PS6.1

ROS signal transduction and cell differentiation in filamentous fungi

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A phosphorelay system coupled to a MAP kinase module is involved in sensing and processing environmental signals in Fungi. In *Aspergillus nidulans*, response regulator (RR) SskA transmits oxidative stress signals to the stress MAPK (SAPK) SakA, which in turns physically interacts with ATF/CREB transcription factor AtfA in the nucleus. This defines a general stress-signalling pathway, which plays differential roles in oxidative stress responses during growth and development. AtfA is needed for the expression of several genes, the conidial accumulation of SakA and the viability of conidia. Furthermore, SakA is active (phosphorylated) in asexual spores, remaining phosphorylated in dormant conidia and becoming dephosphorylated during germination. SakA phosphorylation in spores depends on certain (SskA) but not other (SrrA and NikA) components of the phosphorelay system. Constitutive phosphorylation of SakA prevents both, germ tube formation and nuclear division. Similarly, *Neurospora crassa* SakA orthologue OS-2 is phosphorylated in intact conidia and gets dephosphorylated during germination. We propose that SAPK phosphorylation is a conserved mechanism to regulate transitions between non-growing (spore) and growing (mycelia) states. The *Aspergilli* contain a second SAPK called MpkC. Although *mpkC* mutants are not sensitive to oxidative or osmotic stress, they produce more spores that the wild type strain, suggesting that SakA and MpkC regulate processes related to the production and germination of spores. In addition, to the SakA pathway, RR SrrA and the AP-1 trancription factor NapA are differentially involved in ROS signalling and cell differentiation.

PS6.2