(i) Tim44, a coordinating platform, (ii) the subcomplex Tim14–Tim16, J and J-like proteins regulating the ATPase of mtHsp70, and (iii) Mge1 exchanging ADP vs. ATP on mtHsp70. Structure determination of the Tim14–Tim16 oligomer led to a working hypothesis for the import motor: the Tim14–Tim16 pair switches between two conformations, one in which the HPD motif of Tim14 is available for activating the ATPase domain of mtHsp70 and another one in which the activation is blocked. The switch of the TIM14–Tim16 pair is linked to changing interactions with Tim44 and mtHsp70. The reactions of the various components of the import motor are consistent with a Brownian ratchet type mechanism. In this model, spontaneous oscillations of the unfolded preprotein chain in the import channels of TOM and TIM23 complexes are converted into unidirectional movement by preventing retrograde sliding by regulated transient binding of mtHsp70.

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(S5) Mitochondrial biogenesis symposium abstracts (poster and raised abstracts)

S5.4 Mitochondrial function in cancer cell line models

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Mitochondrial function and respiratory activity is regulated via complex mechanisms that respond to energy status, metabolic elements and stress insults. Elements of mitochondrial dysfunction have been connected to diseases such as metabolic syndromes, cancer and degenerative disorders. The search for biomedical modalities for improvement or stimulation of mitochondrial oxidative activity is therefore considered to be an attractive approach for finding new therapeutic procedures.

The objective of this study was to investigate effects in a selection of cancer cell lines exposed to agents expected to increase mitochondrial biogenesis and respiration. We used pharmacological modulators of glycolysis, respiration and energy status to selectively invoke and facilitate a metabolic shift in the cells towards increased mitochondrial oxidative phosphorylation. Parameters such as cellular content of mitochondria, mitochondrial membrane potential, respiratory rate and glycolytic activity were then analysed. The cell lines exposed different metabolic effects of the treatment, and some cell models were less tolerant than others since the viability was reduced. The metabolic flexibility of the cells seemed to be connected to their ability to thrive under these conditions. This demonstrates that metabolic modulation may have consequences for cell growth and survival, and such approaches may therefore be useful in cancer therapy. Tumours do normally have increased rates of glycolysis combined with reduced respiratory activities, and by targeting this feature it might be possible to develop more selective therapeutic approaches for tumours of different origins.

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S5.5 The trehalose pathway regulates mitochondrial respiratory chain content through hexokinase2 and AMPc IN *Saccharomyces cerevisiae*

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TPS1 encodes for the trehalose-6-P synthase. The trehalose-6-phosphate phosphatase is encoded by TPS2. We studied the respiratory metabolism of both Δtps1 and Δtps2 strains. We show that mutants of the trehalose pathway exhibit modification in the respiratory chain content. In the Δtps1 there is a decrease in the amount of respiratory chains within the cells whereas in the Δtps2 there is an increase in this amount. Because the mitochondrial enzymatic content is modulated through the activity of the Ras/PKA/cAMP pathway, we assessed cAMP content in these strains. There is a good positive correlation between the cellular cytochrome $a+a_3$ content and the cellular cAMP amount. Thus, the effect of the mutations in the trehalose synthesis pathway on mitochondrial enzymatic content is mediated by cAMP level. Furthermore, we investigated the consequences of such mutations on hexokinase 2 deleted strains. In all three hexokinase deleted strains, the mitochondrial amount is comparable to the wild type. Thus, the influence of the tps1 and tps2 deletions on cAMP levels are likely to go through hexokinase.

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S5.6 Reactive oxygen species mediated down-regulation of mitochondrial biogenesis

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Mitochondrial biogenesis necessitates the participation of both the nuclear and the mitochondrial genomes. It is highly regulated and mitochondrial content within a cell varies according to energy demand. In the yeast *Saccharomyces cerevisiae*, the cAMP pathway is involved in the regulation of mitochondrial biogenesis. An overactivation of this pathway leads to an increase in mitochondrial enzymatic content. Out of the three yeast cAMP protein kinases, we have shown that Tpk3p is the one involved in the regulation of mitochondrial biogenesis. Moreover, in the absence of Tpk3p, mitochondria produce large amounts of reactive oxygen species (ROS) that signal to the HAP2/3/4/5 nuclear transcription factors. These transcription factors are well-known to be involved in mitochondrial biogenesis. We clearly establish that an increase in mitochondrial ROS production down-regulates mitochondrial biogenesis. Furthermore, we identified the cysteine of the HAP4 transcription factor that serves the role of sensor of these ROS and is crucial for this signaling. It is the first time that a reactive oxygen specie sensitivity of the transcription factors involved in yeast mitochondrial biogenesis is shown. Such a process could be seen as a mitochondria quality-control process.

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S5.7 Respiratory chain organization in *Neurospora crassa* upon disruption of mitochondrial bc_1 complex

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The mitochondrial bc1 complex (complex III) is a membrane-bound enzyme that catalyses the transfer of electrons from ubiquinol to cytochrome *c*. In mitochondria from many organisms, including the fungus *Neurospora crassa*, dimeric complex III was found associated with complex I. Additional association of complex IV with this core structure leads to the formation of a respirasome. Supercomplexes are thought to ease diffusion and reaction chemistry and may increase individual complex stability. It was recently described for bacteria and mammals that complex III is needed for the assembly/stability of complex I. To elucidate the role of complex III in the organization of the respiratory chain of *N. crassa,* we analyzed strains devoid of either the rieske iron sulfur or the core II proteins. The supramolecular organization of the oxidative phosphorylation system was characterized through BN-PAGE, 2D BN/BN-PAGE and 2D BN/SDS-PAGE and the efficiency of the respiratory chain analysed by oxygen consumption measurements. The results obtained indicate that absence of complex III activity (i) is not associated with the absence of complex I, as suggested for other organisms, (ii) leads to the induction of the alternative oxidase and (iii) results in the re-organization of the respiratory chain with the establishment of different interactions between the complexes.

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S5.8 Biogenesis of the mitochondrial carrier translocase

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Approximately 99% of all mitochondrial proteins are synthesized in the cytosol and have to be imported into mitochondria. Sophisticated machineries mediate targeting, import, and assembly into one of the four mitochondrial subcompartments: outer membrane, intermembrane space, inner membrane, and matrix. The TIM22 complex (translocase of the inner mitochondrial membrane 22) or carrier translocase is the final assembly machinery for metabolite carriers, a class of polytopic membrane proteins with multiple internal targeting sequences. All subunits of the TIM22 complex are nuclear-encoded and require mitochondrial translocase machineries for their import and assembly into the functional complex. Three out of six subunits of this complex, Tim18, Tim22, and Tim54, are integral membrane proteins and form the core of the translocase with the protein-conducting channel. Our aim is to understand the biogenesis of these core subunits, with respect to import routes and assembly into the TIM22 complex. We use in vitro import assays with radiolabeled Tim18, Tim22, and Tim54 precursors and mitochondria isolated from mutant strains. Analysis by SDS-PAGE and blue-native electrophoresis revealed a different assembly pathway for each subunit. We could show that insertion of Tim18, Tim22, and Tim54 into the inner membrane and assembly into the mature TIM22 complex are independent events with distinct requirements.

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S5.9 Biochemistry and physiology of the mitochondrial serine protease LACTB

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Mammalian mitochondria harbor a conserved 60 kDa protein called LACTB that has evolved from bacterial peptidoglycan-synthesizing enzymes. Phylogenetic analysis indicates that LACTB is closely related to low molecular weight penicillin-binding proteins class B. We have purified LACTB from rat liver mitochondria for biochemical analysis. Submitochondrial fractionation and immunoelectron microscopy showed that LACTB is localized in the mitochondrial intermembrane space. Plasmid constructs of LACTB with or without the predicted mitochondrial import pre-sequence confirmed that mitochondrial import of LACTB is dependent on the N-terminal amino acid sequence. MALDI-TOF-TOF analysis of endogenous rat liver LACTB revealed a common N-terminal tetrapeptide motif that is also found in a set of apoptosis-inducing proteins. 2D blue native SDS-PAGE demonstrated that LACTB migrates at an apparent molecular weight of >600 kDa. Separation of mitochondrial intermembrane space proteins by gradient centrifugation followed by visualization of proteins in the LACTB-containing fraction by transmission electron microscopy revealed the presence of filamentous structures. Our data suggest (i) that LACTB is part of a large protein complex and (ii) that LACTB can interact with the proteins involved in the apoptotic signal transduction pathway through its N-terminal tetrapeptide motif.

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S5.10 Unraveling the physiological function of the mitochondrial *i*-AAA protease Yme1

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ATP-dependent AAA^+-(ATPases Associated with various cellular Activities) proteases are ubiquitously expressed proteolytic machines essential for the control of many regulatory proteins and maintenance of protein quality. Two mitochondrial representatives of this family are the *i*- and *m*-AAA-protease, anchored to the inner membrane of mitochondria. Both proteases are conserved in all eukaryotic species and show versatile functions within mitochondria. They accomplish the quality control of mitochondrial protein, but in addition AAA-proteases are involved in the dislocation of proteins from the inner membrane of mitochondria, processing of proteins and their import into mitochondria. Along with these functions different phenotypes are linked to the loss of AAA-proteases, including axonal degeneration in mammals. Phenotypes associated with the loss of the *m*-AAA-protease in yeast can be explained by an impaired processing of a mitochondrial ribosomal subunit and defective synthesis of mitochondrially encoded respiratory chain subunits. In contrast, phenotypes of cells harbouring a deletion of the *i*-AAA-protease are not understood. We therefore used different approaches to define the function of Yme1 in mitochondria. In the first approach, we employed a His-tagged version of a proteolytically inactive variant of the *i*-AAA-protease subunit Yme1 as a substrate trap for affinity purification. Furthermore, we screened for synthetic lethal interactions of *YME1*, in order to identify pathways in which the Yme1 protein might be crucial. The recent findings we achieved, using these two methods will be discussed.

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