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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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