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multisystem disease. The deletion of 18-base pair (np 15649-15666) causes the loss of six aminoacids in the sixth TM helix of the protein, leaving the remaining of the cytochrome b sequence in frame. From patient's fibroblasts cybrid cell lines were generated and syngenic clones with different mutation load were selected. The cell viability in galactose medium, the activity and assembly of CIII were reduced depending on the mutation load, thus unequivocally establishing its pathogenic role. In addition, we show that the amounts of the supercomplexes III2IV1 and I1III2IVn were reduced, indicating a progressive loss of CIII and CIV interactions. Conversely, a very large supercomplex containing CI, CIII and CIV was apparent in heteroplasmic cybrids, suggesting that a mixture of mutant and wild type CIII could form different and stronger interactions with CI and CIV. Finally, the CI + CIII integrated activity as well as the CI-driven ATP synthesis were highly conserved despite progressive CIII deficiency, supporting our hypothesis that the dynamic organization of respiratory supercomplexes can mitigate their dysfunction. Supported by grants from PRIN (MR) and NIH (GM 38237 to FD).

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S5.P1

New players involved in regulation of micos complex function and stability

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Mitochondria are essential organelles for every eukaryotic cell. Four mitochondrial compartments can be distinguished: outer membrane, inner membrane, intermembrane space and matrix. The mitochondrial inner membrane consists of the inner boundary membrane connected with the cristae membrane by the crista junctions. MICOS, a recently discovered protein complex, is crucial for the establishment and maintaining of proper inner membrane architecture. Furthermore, MICOS components were reported to interact with translocase of the outer membrane (TOM) and Mia40 oxidoreductase to facilitate transport of intermembrane space precursor proteins. Therefore, MICOS is also involved in the control of mitochondrial protein biogenesis. It remains to be discovered how the MICOS complex is assembled and regulated. Sco1 and Cox17 are mitochondrial proteins that are involved in the assembly of cytochrome c oxidase. Here, we report an interplay between these two proteins and the MICOS complex. This study aims to reveal new players involved in maintaining the architecture and function of the inner mitochondrial membrane.

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S5.P2

The precursor of subunit III of cytochrome c oxidase is imported, processed and assembled into isolated mitochondria from the alga Polytomella sp

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Cytochrome c oxidase (Complex IV) is the terminal electron acceptor of the mitochondrial respiratory chain. The membrane-bound complex is constituted by both mitochondria-encoded and nucleus-encoded polypeptides. The three subunits that form the structural core of the enzyme, Cox1, Cox2 and Cox3, are encoded in the mitochondrial genome, synthesized in the matrix and integrated directly into the mitochondrial inner membrane. Nevertheless, in chlorophycean algae like *Chlamydomonas reinhardtii*, *Volvox carteri* and the colorless alga *Polytomella* sp., Cox3 is encoded in the nucleus and its precursor protein is synthesized in the cytosol with a long, cleavable, N-terminal mitochondrial targeting sequence (MTS) of around 100 residues. Therefore, the algal mitochondria must import the Cox3 polypeptide and integrate it to the mitochondrial inner membrane and into Complex IV. Here, we studied the in vitro import of the algal Cox3 precursor by isolated, import-competent mitochondria of *Polytomella* sp. It was found that Cox3 is readily imported into mitochondria in a process that requires an energized inner membrane, and once it is exposed to the matrix, it is proteolitically processed to its mature form. When an import time course was carried out, a transient Cox3 intermediate was identified, suggesting that the long MTS is processed more than once. The first cleavage is sensitive to the metalo-protease inhibitor 1,10-ortophenantroline, suggesting that this step is probably carried out by the matrix-located Mitochondrial Processing Protease. The mature Cox3 polypeptide became resistant to carbonate extraction, indicating that it had integrated into the inner mitochondrial membrane. Furthermore, the imported Cox3 protein was shown to assemble into cytochrome c oxidase, as judged by the presence of a labeled band co-migrating with Complex IV in Blue Native Electrophoresis. To our knowledge, this is the first time that the in vitro mitochondrial import of a cytosol-synthesized subunit III of cytochrome c oxidase is described. We propose a model for the biogenesis of Cox3 in chlorophycean algae.

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S5.P3

Subunit NUMM (NDUFS6) plays a key role in the late stage of complex I assembly

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In addition to its fourteen central subunits harboring the bioenergetic core functions, mitochondrial complex I consists of a large number of accessory subunits acquired during evolution [1,2]. Assembly of the largest and most complicated multiprotein complex of the respiratory chain proceeds via intermediates and is supported by a number of assembly factors [3]. Here we studied accessory subunit NUMM of complex I from the aerobic yeast *Yarrowia lipolytica* (bovine 13 kDa, human NDUFS6) that harbors a conserved zinc binding motif comprising 3 Cys and 1 His. Purification and biochemical characterization of an assembly intermediate from numm Δ deletion strain as well as mutagenesis of the metal binding site shed light on the specific function of NUMM in complex I assembly and its interplay with assembly factor N7BML (B17.2L; NDUFAF2). Interestingly, a change of one of the conserved cysteine residues in the human NDUFS6 subunit (corresponding to C128 in NUMM of Y. lipolytica) into tyrosine was found to affect complex I assembly and to cause fatal neonatal lactic acidosis [4].