restore the assembly of complex III in yeast cells mutated in the AAA domain of Bcs1. Unexpectedly, the first class of compensatory mutations mainly target the mitochondrial ATP synthase, leading to a strong decrease in the ATP hydrolysis activity while maintaining a sufficient level of ATP synthesis to sustain respiratory growth. We propose that by reducing ATP hydrolysis by the ATP synthase, the compensatory mutations increase the concentration of ATP in mitochondria, thereby increasing the ATP hydrolysis activity of the mutated Bcs1p and allowing it to recover its chaperon function.

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S5.L4

Molecular bases for the complex I stability dependency from other respiratory complexes

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In mammalian mitochondria, complex I becomes unstable in the absence of complexes III (1) or IV (2, 3). On the contrary, the absence of complex I does not affect substantially the stability of the other respiratory complexes. By systematic analysis of the degradation process of complex I in cellular models of CIII or CIV ablation, we have identified the signals that trigger it, and defined the molecular mechanism responsible for the stability of complex I. Finally we have investigated the potential physiological role of this interdependency.

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S5.O1

High molecular weight forms of mammalian respiratory chain complex II

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Supercomplexes of mammalian mitochondrial respiratory chain formed by complexes I, III, and IV are well established. In contrast, the involvement of succinate dehydrogenase, complex II (CII), linking respiratory chain with tricarboxylic acid (TCA) cycle, in supramolecular structures remains questionable. To search for higher molecular weight forms of CII and specific interactions of CII with other complexes of oxidative phosphorylation (OXPHOS) pathway or with TCA cycle we combined mild detergent solubilisation of mitochondria and different types of native electrophoresis. For experiments we used rat tissues and different cell lines of murine and human origin including fibroblasts with different OXPHOS defects or cells devoid of mtDNA. Mitochondrial proteins were solubilised with digitonin, separated by native electrophoresis or two-dimensional electrophoretic systems and detected by immunoblotting or in-gel assay of activities of OXPHOS complexes. For immunoprecipitation of CII and ATP synthase (complex V, CV) we used antibodies to SDHA subunit of CII and antibodies to F1 subunits of CV.

We have found that digitonin-solubilised complex II quantitatively forms high molecular weight structures (CII_{hmw}) that can be resolved by clear native electrophoresis. CII_{hmw} structures are enzymatically active and differ in electrophoretic mobility between tissues (500–over 1000 kDa) and cultured cells (400–670 kDa). Whilst their formation is unaffected by isolated defects in other respiratory chain complexes, they are destabilised in mtDNA-depleted rho0 cells. Molecular interactions responsible for the assembly of CII_{hmw} are rather weak with the complexes being more stable in tissues than in cultured cells. Whilst our electrophoretic studies and immunoprecipitation experiments of CII_{hmw} do not indicate specific interactions with the respiratory chain complex I, III or IV or enzymes of the tricarboxylic acid cycle, they point out to a specific interaction between CII and ATP synthase [1].

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S5.O2

Impaired mitochondrial energetic function and altered supramolecular interactions of respiratory chain complexes in cells bearing a novel pathogenic cytochrome b microdeletion

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Cytochrome b is the only mtDNA-encoded subunit of the respiratory complex III (ubiquinol: cytochrome c oxidoreductase; CIII), the central component of the respiratory chain. In its native form, CIII is dimeric and is closely associated in varying proportions with CI and CIV to form supramolecular structures, referred to as supercomplexes or "respirasomes". The occurrence of such supercomplexes as structural and functional entities is well documented. This indicates that interactions among CI/CIII and CIII/CIV are dynamic events, allowing cells to adapt their respiratory activity to specific cell-type requirements. This concept is also important in relation to human diseases caused by mitochondrial dysfunctions. Defects in CIII are relatively rare and mostly associated with mutations in the MTCYB gene. It has been shown that mutations in this gene can result in CIII deficiency alone or can produce combined CI and CIII failure, likely as a consequence of the critical role of CIII in the stability of CI in respirasomes. Here, we report a novel heteroplasmic mtDNA micro-deletion in MTCYB gene, identified in a patient suffering a