systemic disorders of variable severity. Due to the complex III dual genetic origin, these enzyme defects can be attributed to mutations located either in mitochondrial or in nuclear genes. An increasing number of specific regulatory proteins involved in the biosynthesis of this respiratory chain complex that may also lead to complex III deficiency has been described. One of these proteins is BCS1L, an assembly factor that facilitates the insertion of the catalytic Rieske Iron–Sulfur subunit into respiratory chain complex III. Mutations in the *BCS1L* gene constitue the main cause of complex III deficiency. In the search for potential biomarkers for complex III deficiency we performed two-dimensional difference gel electrophoresis (2D DIGE) followed by spot picking and subsequent protein digestion for mass spectrometry (MS) identification in fibroblasts from four complex III-deficient patients harboring mutations in the *BCS1L* gene and four independent controls. Here we report 41 proteins that are differentially expressed in patients harbouring *BCS1L* mutations compared to controls. These proteins are mainly related to energy metabolism, cytoskeleton maintenance processes, regulation of DNA transcription and replication and protein synthesis, cell cycle regulation and oxidative stress response. Further studies are required for the validation and functional characterization of possible biomarkers that may be of importance for future diagnostic, therapeutic, and prognostic approaches in mitochondrial disorders.

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18P5

Structural interactions of mitochondrial ATP synthasome components

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The mitochondrial phosphorylation apparatus consists of three protein components — ADP/ATP carrier (AAC), inorganic phosphate carrier (PiC), and F1Fo-ATP synthase. According to some experimental evidence, these proteins of the inner mitochondrial membrane could interact not only functionally, but also structurally [1]. Their supercomplex called ATP synthasome would show better catalytic efficiency of ATP synthesis.

To characterize interactions of AAC, PiC, and ATP synthase in mammalian cells, we used two models of isolated ATP synthase deficiency — rat brown adipose tissue with physiological deficiency of ATP synthase due to low expression of Fo-c subunit and fibroblast cultures of patients with ATP synthase deficiency caused by a mutation in the assembly factor TMEM70. SDS-PAGE/WB analysis of isolated mitochondria revealed that among rat tissues, AAC and PiC are most abundant in the tissues with high energetic demands, i.e. in heart and skeletal muscle. However, their amount was also high in brown adipose tissue despite the low content of ATP synthase. In the ATP synthase deficient patients' fibroblasts, we also found a high content of AAC and PiC that was even increased in comparison to control cells. The lack of correlation in the contents of ATP synthase and mitochondrial carriers AAC and PiC does not support a direct relationship in the regulation of their biogenesis.

Simultaneously, we studied structural interactions of AAC, PiC, and ATP synthase using various approaches. Detergent-solubilized mitochondria were used for immunoprecipitation and native and two-dimensional gel electrophoreses combined with either immunodetection with specific antibodies or MS analysis. Our results indicate that both carriers interact with monomeric and oligomeric forms of ATP synthase. The majority of these carriers, however, are present out of ATP synthasome, probably as dimers.

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18P6

Mitochondrial complex I plays an essential role in human respirasome assembly

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The biogenesis and function of the mitochondrial respiratory chain (RC) involves the association of individual RC enzyme complexes into supercomplexes or respirasomes, through a biosynthetic process that remains largely unknown. Here we show that in human cells, I + III2 + IVn supercomplexes do not originate from the association of fully-assembled individual holoenzymes. Rather, we demonstrate that respirasome biogenesis involves a complex I assembly intermediate acting as a scaffold for the combined incorporation of free subunits and subcomplexes from complexes III and IV. The process ends with the association of the complex I NADH dehydrogenase catalytic module, which leads to complex I and respirasome activation. Our studies reveal that while complexes III and IV can assemble either as free holoenzymes or by incorporation of free subunits into supercomplexes, the respirasomes constitute the structural units where complex I is assembled and activated, thus explaining the significance of the respirasomes for RC function.

Reference

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18P7

FRET reveals lactacystin-induced increase of Tom20/TOM assembly

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The Translocase of the Outer mitochondrial Membrane (TOM) is a protein complex that translocates nuclear encoded proteins into mitochondria. The isolated TOM holo complex consists of the pore-forming channel protein Tom40, Tom22 and the small Tom proteins