Abstract:

The complexes of oxidative phosphorylation (OXPHOS) are situated in the inner mitochondrial membrane in higher structural and functional complexes, so-called supercomplexes, which facilitates substrate channeling. ATP synthase is also able to organize in higher structures. In mammalian mitochondria, ATP synthase is usually present in a dimeric form. There is evidence of its trimerization and even tetramerization. Furthermore, it seems that ATP synthase catalyzing the phosphorylation of ADP to ATP, adenine nucleotide translocator (ANT) ensuring the exchange of ADP for newly synthesized ATP across the inner mitochondrial membrane and phosphate carrier (PiC) allowing the import of inorganic phosphate (P_i) into the matrix of mitochondria are assembled in a supercomplex called ATP synthasome. This association among the components of phosphorylative apparatus seems to increase the efficiency of processes leading to the ATP synthesis.

First, we studied amounts of the components of phosphorylative apparatus in connection with various ATP synthase contents among mitochondria isolated from nine rat tissues. Mitochondrial proteins were separated by denaturing electrophoresis (SDS-PAGE) and their content was analyzed using specific antibodies. In agreement with our expectations, the highest content of phosphorylative apparatus components as well as of cytochrome *c* oxidase, representing OXPHOS complexes, was found in heart and skeletal muscle mitochondria, ergo in tissues with high energetic demands. Surprisingly, the content of ANT and PiC was high also in brown adipose tissue despite physiologically reduced biogenesis of ATP synthase. Apparently, the amounts of translocators are affected by the content of ATP synthase neither in rat tissues nor in cell cultures with various genetic defects of ATP synthase (mutations in the nuclear genes *TMEM70* and *ATP5E*, coding for an assembly factor and $F_1\varepsilon$ subunit of ATP synthase, respectively, and in the mitochondrial gene *ATP6* coding for F_oa subunit). Some of these ATP synthase defects lead to even increased contents of translocators in comparison to control cells.

Structural associations of phosphorylative apparatus components were studied by solubilization of mitochondria using mild detergents. Supposed interactions and the existence of ATP synthasome were shown by various native and two-dimensional electrophoreses. Mass-spectrometric analysis of native gel pieces also confirmed co-localization of ATP synthase, ANT, and PiC. However, only fractions of contents of ATP synthase and both

translocators associate to form ATP synthasome. The majority of translocators exist out of ATP synthasome, probably in dimeric forms.

Key words: ATP synthase, ATP synthasome, ANT, BAT, electrophoresis, OXPHOS, PiC, solubilization, supercomplexes, TMEM70