Assembly of F_o part subunits into mammalian ATP synthase

Tauchmannová Kateřina, Pecina Petr, Nůsková Hana, Ho Hien, Kovalčíková Jana, Mráček Tomáš, Houštěk Josef

Institute of Physiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic

E-mail: Katerina.Tauchmannova@fgu.cas.cz

The biogenesis of mammalian ATP synthase is complex process believed to proceed via several modules. It starts with the formation of F_1 catalytic part, which is in the later steps connected with the membranous subcomplex. The final phase is represented by incorporation of the two mtDNA-encoded subunits F_o-a and A6L. However, little is known about the position of two newly described F_o accessory subunits DAPIT (also termed Usmg5) and MLQ (also known as c14orf2) in the assembly scheme and about their role in regulation of ATP synthase biogenesis. To resolve this, we have utilised several model systems, namely rho^0 cells lacking mtDNA and thus both subunits F_o-a and A6L, cells harbouring 9205delTA microdeletion, which results in the absence of the subunit F_o-a, HEK293 cells with knockdown of DAPIT protein and HEK293 cells with knockout of MLQ protein and followed the assembly state of ATP synthase among them.

Contrary to previously reported data, we observed normal levels of assembled ATP synthase in DAPIT knockdown and MLQ knockout cells. Our results indicate, that lack of DAPIT protein leads to the assembly of more labile, but complete and functional enzyme. Absence of either F_o-a alone or F_o-a and A6L results into the normal levels of structurally altered, labile, and ~60 kDa smaller enzyme complex, which also lacks DAPIT and MLQ. This complex retains the ATP hydrolytic activity but is unable to synthesize ATP. Cells with the MLQ knockout presented with the phenotype similar to the lack of F_o-a: normal content of smaller and labile complex. In the absence of MLQ, ATP synthase did not contain also subunits F_o-a and A6L. This complex also retained ATP hydrolytic activity, while its phosphorylating capacity was affected. In all the cell lines tested we found that the total amount of individual subunits corresponds well with the amount of the subunit, associated with fully assembled ATP synthase complex. This suggests, that subunits that are not incorporated into the complex, are degraded in the cell. This hypothesis is supported by the fact, that in the cells lacking subunit MLQ the biosynthesis of both mtDNA-encoded subunits F_o-a and A6L is decreased, and these subunits are fast degraded.

Based on our data, we conclude that MLQ and F_o-a closely associate and their incorporation into the enzyme complex depends on each another. On the contrary, DAPIT protein seems to be incorporated at the very last step and its presence stabilises the holoenzyme.

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