Novel factors essential for human mitochondrial F$_0$F$_1$-ATP synthase activity found by MASC (Mitochondrial Activity of SLO-permeabilized Cells) screening

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We and other groups have studied the regulatory mechanism of human F$_0$F$_1$-ATP synthase, especially, mechanism related to IF1 (inhibitor of F$_1$) [1]. It is thought that IF1 in mammalian mitochondria inhibits ATP hydrolysis when cells encounter conditions unfavorable for synthesis such as ischemia. Factors other than IF1 may be also involved in the regulation of human F$_0$F$_1$-ATP synthase activity as demonstrated by the recent reports on the effect of PKC delta and TMEM70. However, collective screening of the regulatory factors of mitochondrial ATP synthesis has not been done mainly because there is no high throughput assay for mammalian F$_0$F$_1$-ATP synthase activity. Conventional protocol to measure F$_0$F$_1$ activity requires sub-cellular fractionation from large amounts of culture cells. Furthermore, the purified mitochondrial fractions should be used in short time due to rapid decay of the ATP synthesis activity. We invented new assay method for the mitochondrial ATP synthase activity of human culture cells. We utilize streptolysin O (SLO) to make pores in plasma membrane of host cells without damaging organelle membranes. Cytosolic components are washed out and mitochondrial ATP synthesis activity is measured (Biochem. Biophys. Res. Commun., 2010, 401 (4) 538–543). The new method, MASC (Mitochondrial Activity of SLO-permeabilized Cells) assay, enables us to directly measure ATP synthesis activity of F$_0$F$_1$ of culture cells on 96-well plate, suitable for high throughput screening for factors that affect ATP synthesis. First, factors assumed to be interacting with F$_0$F$_1$-ATP synthase were knocked down and screened. By this screening, DAPT (J. Biol. Chem., 2011, 286 (23) 20292–20296) was found to be essential for ATP synthesis of F$_0$F$_1$. Next, unknown genes coding mitochondrial localized proteins were knocked down and screened. By this screening, a critical gene for F$_0$F$_1$ activity was found. To summarize, we established a new screening method for human F$_0$F$_1$-ATP synthase activity and found new factors that are essential for ATP synthesis activity.


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Conformational changes of the ε-subunit of the H$^+$-ATP synthase by binding of nucleotides

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Movements of the ε-subunit of the H$^+$-ATP synthase from Escherichia coli (EF$_0$F$_1$) are investigated by single molecule spectroscopy. Two mutants of EF$_0$F$_1$ that differ in the binding site of the fluorescent dye on the ε-subunit (εA93C and εY114C) have been used. Both mutants have the same binding site on the γ-subunit (γT106C). The ε- and the γ-subunits of EF$_0$F$_1$ have been covalently labeled with fluorescent dyes. Depending on the investigated distances two flourescent pairs, ATTO 532/ATTO 610 or ATTO 532/ATTO 647N, have been used. The labeled EF$_0$F$_1$ is integrated into liposomes. Single-pair fluorescence resonance energy transfer is measured in freely diffusing proteoliposomes with a confocal two channel microscope. It has been shown with both mutants that addition of the nucleotide ADP changes the conformation of the ε-subunit from the “up” conformation to the “up extended” conformation. Addition of the nucleotides ATP or AMPPNP changes the “up” conformation to the “down” conformation.

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The interaction of the diarylquinoline TMC207, a new tuberculosis antibiotic, with its target mycobacterial ATP synthase

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