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Novel factors essential for human mitochondrial F_oF_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_oF_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_oF_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_oF_1-ATP synthase activity. Conventional protocol to measure F_oF_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 synthesis 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_oF_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_oF_1-ATP synthase were knocked down and screened. By this screening, DAPIT (*J. Biol. Chem.*, 2011, *286* (23) 20292– 20296) was found to be essential for ATP synthesis of F_oF_1. Next, unknown genes coding mitochondrially localized proteins were knocked down and screened. By this screening, a critical gene for F_oF_1 activity was found. To summarize, we established a new screening method for human F_oF_1-ATP synthase activity and found new factors that are essential for ATP synthesis activity.

[1] *J. Biol. Chem.*, 2012, **287** (22) 18781-7

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Characterization of CyPD - F_OF_1ATP synthase interaction: Role of the OSCP subunit

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The mitochondrial F_OF_1ATP synthase is a large multisubunit complex of 600 kDa anchored to the inner mitochondrial membrane, where it catalyzes ATP synthesis or hydrolysis at high and low proton electrochemical gradient, respectively. We have previously demonstrated that Cyclophilin D (CyPD, the only known mitochondrial immunophilin) binds the lateral stalk and inhibits the ATP synthase. The exact binding site in the lateral stalk and the mechanism(s) through which CyPD modulates ATP-synthesis/hydrolysis have not been defined yet. Here we studied the association of CyPD to ATP synthase in bovine, human and mouse heart mitochondria in order to define the site and mechanism of interaction. We found that OSCP subunit is the binding site for CyPD; consistently (i) decreasing the amount of OSCP subunit by RNA interference also reduced CyPD association to the ATP synthase; (ii) Benzodiazepine 423 (Bz423, an OSCP subunit-targeted inhibitor of F_OF_1ATP synthase) inhibited ATP hydrolysis both in wild-type and in CyPD-null mitochondria, yet higher Bz423 concentrations were required to inhibit the ATP synthase in wild-type mitochondria, where the drug displaced CyPD from the enzyme complex. Since Bz423 binds OSCP helices 3 and 4, these data suggest that CyPD binding specifically affects this site on the ATP synthase. Consistent with a site-specific effect of Bz423, no difference in inhibitory titer between wild-type and CyPD-null mitochondria was observed with oligomycin (which inhibits the ATP synthase at subunit a) and resveratrol (which binds the α/β subunits). Like binding of Bz423 to OSCP, the interaction between CyPD and the lateral stalk of the ATP synthase was disrupted by increasing ionic strength, suggesting that the interaction itself is electrostatic in nature.

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Conformational changes of the ε-subunit of the H^+-ATPsynthase by binding of nucleotides

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Movements of the ε-subunit of the H^+-ATPsynthase from *Escherichia coli* (EF_OF_1) are investigated by single molecule spectroscopy. Two mutants of EF_OF_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_OF_1 have been covalently labeled with fluorescent dyes. Depending on the investigated distances two fluorescents pairs, ATTO 532/ATTO 610 or ATTO 532/ATTO 647N, have been used. The labeled EF_OF_1 is integrated into liposomes. Single-pair fluo rescence 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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