Stepwise rotational movements of the $H^+$-ATP synthase $\gamma$-subunit have been observed in several independent studies. To test if the stepping rotation involves the entire length of the $\gamma$-subunit, or whether the $\gamma$-subunit possesses a rotational bearing we genetically modified the mitochondrial $H^+$-ATP synthase from yeast (Saccharomyces cerevisiae, YRD15), M$_{Fo}$F$_1$, for fluorescence spectroscopic investigations: A fluorescent protein (Enhanced Green Fluorescent Protein, EGFP) was attached to the $\gamma$-subunit C-terminus via an anti-parallel leucine-zipper helix in order to mechanically couple a fluorescent marker to movements of this domain. For production of a functional fusion protein the genetic modification was introduced via genomic integration and cells were subsequently grown with ethanol as a carbon source. The EGFP- M$_{Fo}$F$_1$ fusion protein was isolated, purified and characterized. In addition to EGFP at the $\gamma$-subunit cysteines were introduced either in OSCP-subunit or in the b-subunit. This allows specific labeling of these subunits with fluorophores carrying a maleimide group. Fluorescence Resonance Energy Transfer (FRET) was measured with these labeled enzymes using molecule ensembles and with single molecules.

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Hypothetical trypanosoma protein may help anchor the F$_1$-ATPase moiety to the mitochondrial membrane

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The unicellular flagellate, Trypanosoma brucei, is a medically important parasite that infects humans and livestock. Interestingly, the mitochondrial (mt) F$_{0}$F$_{1}$-ATPase activity is essential in the infectious form of this pathogen as it hydrolyzes ATP to pump protons into the mt inner membrane space to maintain the mitochondrial membrane potential (mt $\Delta \Psi$) in the presence of a traditional cytochrome mediated respiratory chain. Unlike the well conserved higher eukaryotic F$_{0}$F$_{1}$-ATP synthases, the T. brucei F$_{0}$F$_{1}$-ATP synthase complex contains several trypanosoma specific subunits with unknown function. RNAi silencing of one the largest novel subunits, Tb2930 (40kDa), led to a significant decrease of the mt $\Delta \Psi$ and consequently to a major growth phenotype, indicating that the F$_{0}$F$_{1}$-ATPase is not functioning properly even though its structural integrity seems unchanged. Since this hypothetical subunit is membrane-bound and co-sediments with both monomeric and multimeric assemblies of the F$_{0}$F$_{1}$-ATPase complex in glycerol gradients, we propose that Tb2930 might be part of the membrane-bound F$_{0}$ moiety or part of the membrane-bound peripheral stalk.

To further explore the function of this intriguing protein, we silenced the expression of Tb2930 in a strain of trypanosoma lacking mitochondrial DNA and thus subunit a, an essential component of the F$_{0}$ moiety and proton pore. These cells maintain their mt $\Delta \Psi$ by the electrogenic exchange of ATP$^+$/ADP$^3$ by the ATP/ADP carrier (AAC) and the hydrolytic activity of the F$_{1}$-ATPase moiety. Importantly, the depletion of Tb2930 resulted in a significant growth phenotype caused by a decreased mt $\Delta \Psi$, highlighting its function in the F$_{1}$-ATPase/AAC maintained mt $\Delta \Psi$. We are currently exploring the possibility that Tb2930 is responsible for connecting the F$_{1}$-ATPase moiety to the mitochondrial membrane in the absence of the F$_{0}$ moiety, thus increasing the efficiency of the functional association between F$_{1}$-ATPase and AAC.

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Screening of protein kinase inhibitors that affect ATP synthesis activity using MASC assay

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In mammal, mitochondrial F$_{0}$F$_{1}$-ATP synthase (F$_{0}$F$_{1}$) produces most of cellular ATP under aerobic conditions. The activity of F$_{0}$F$_{1}$ must be regulated in response to cellular energy demands, but regulatory mechanisms remain largely unknown. Recently, signaling pathways involved in energy metabolism, e.g., glucose and lipid metabolism, have been reported. In addition, several groups reported that subunits of F$_{0}$F$_{1}$ were phosphorylated by protein kinases, allowing the speculation that mitochondrial ATP synthesis activity is regulated by protein kinase. In this study, we performed screening for mitochondrial ATP synthesis in human cultured cell treated with various protein kinase inhibitors. HeLa cells were treated with each of 80 inhibitors and after 18-hr incubation, mitochondrial ATP synthesis rate was measured by MASC (Mitochondrial Activity of Storeptolysin O-permeabilized Cell) assay, a novel method developed by Fujikawa, et al [1]. Twelve inhibitors were found to reduce mitochondrial ATP synthesis rate but eight of them have direct effect on cell viability and mitochondrial membrane potential. The remaining four kinase inhibitors inhibits ATP synthesis without affecting cell viability and without dissipating mitochondrial membrane potential, indicating that they have no uncoupler action and respiratory chain is not damaged. When the target kinases of these four inhibitors were knocked-down by RNA interference, ATP synthesis rate was reduced. Native-PAGE followed by western blotting against anti-F$_{0}$F$_{1}$ antibody revealed that the amount of F$_{0}$F$_{1}$-ATP synthase in smMLCK (smooth muscle type Myosin light chain kinase) knock-down cells was decreased. In addition, growth of smMLCK knock-down cells was significantly decreased under glucose-deprivation condition. These results suggest that smMLCK is involved in regulation of ATP production by F$_{0}$F$_{1}$-ATP synthase in mitochondria (but not glycolysis). Thus, we found that some protein kinases influence the activity of F$_{0}$F$_{1}$-ATP synthase and smMLCK participates in expression or assembly of F$_{0}$F$_{1}$-ATP synthase.

Reference


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Conformational change of the $\gamma$ subunit regulates the ATP hydrolysis activity of cyanobacterial ATP synthase

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The ATP synthase catalyzes synthesis of ATP from ADP and inorganic phosphate by using the transmembrane electrochemical potential of protons. The catalytic core of ATP synthase, the $\alpha_1\beta_1\gamma$