Stepwise rotational movements of the H\(^+\)-ATP synthase γ-subunit have been observed in several independent studies. To test if the stepping rotation involves the entire length of the γ-subunit, or whether the γ-subunit possesses a rotational bearing we genetically modified the mitochondrial H\(^+\)-ATP synthase from yeast (Saccharomyces cerevisiae, YRD15), Mf\(_2\)F\(_1\), for fluorescence spectroscopic investigations: A fluorescent protein (Enhanced Green Fluorescent Protein, EGFP) was attached to the γ-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-Mf\(_2\)F\(_1\) fusion protein was isolated, purified and characterized. In addition to EGFP on the γ-subunit cytostines 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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IP41

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\(_2\)F\(_1\)-ATPase activity is essential to the infectious pathogenic parasite that infects humans and livestock. Interestingly, the E-mail: 370 05 České Budějovice, Czech Republic

Although the well conserved higher eukaryotic FoF\(_1\)-ATP synthases, the T. brucei F\(_2\)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 ΔΨ and consequently to a major growth phenotype, indicating that the F\(_2\)-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\(_2\)F\(_1\)-ATPase complex in glycerol gradients, we propose that Tb2930 might be part of the membrane-bound F\(_2\) 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 trypansoma lacking mitochondrial DNA and thus subunit a, an essential component of the F\(_2\) moiety and proton pore. These cells maintain their mt ΔΨ by the electrogenic exchange of ATP\(^3-\)/ADP\(^-\) 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 ΔΨ, highlighting its function in the F\(_1\)-ATPase/AAC maintained mt ΔΨ. 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 Fo moiety, thus increasing the efficiency of the functional association between F\(_1\)-ATPase and AAC.

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IP42

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

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In mammal, mitochondrial F\(_2\)F\(_1\)-ATP synthase (F\(_2\)F\(_1\)) produces most of cellular ATP under aerobic conditions. The activity of F\(_2\)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\(_2\)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\(_2\)-F\(_1\) antibody revealed that the amount of F\(_2\)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\(_2\)F\(_1\)-ATP synthase in mitochondria (but not glycolysis). Thus, we found that some protein kinases influences the activity of F\(_2\)F\(_1\)-ATP synthase and smMLCK participates in expression or assembly of F\(_2\)F\(_1\)-ATP synthase.

Reference


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IP43

Conformational change of the γ 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 αβγ3ειϕγ

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complex, has a hexagonal arrangement consisting of an $\alpha_3\beta_3$ ring and the $\gamma$ subunit [1]. The $\gamma$ subunit is a central shaft of this motor enzyme and has a coiled-coil structure of N- and C-terminal $\alpha$-helices and a globular domain. In the case of cyanobacterial and chloroplast ATP synthase, the $\gamma$ subunit has a unique 30-40 amino acid sequence in this globular domain. In the previous study, we prepared the mutant $\alpha_3\beta_3\gamma$ complex of cyanobacterial ATP synthase whose inserted sequence was deleted [2,3]. Although the insertion is far from catalytic sites, the mutant complex shows a remarkable increase in ATP hydrolysis activity. We revealed that this activation was caused by a reduced tendency to lapse into ADP inhibition. Based on this study, we postulated that deletion of the insertion induces a conformational change of the $\gamma$ subunit that results in a change of the activity. To prove the hypothesis, we prepared a mutant complex which can lock the possible conformational change by a disulfide bond formation. Consequently, the obtained mutant showed a significant change in ATP hydrolysis activity by the disulfide bond formation. Highly active locked complex was insensitive to LDAO, suggesting that the complex is resistant to ADP inhibition. In addition, the lock of conformational change affected $\varepsilon$ inhibition. These results strongly suggest that the conformational change in the $\gamma$ subunit can regulate the activity by changing both ADP inhibition and $\varepsilon$ inhibition, which are thought to be regulatory mechanisms to prevent a wasteful ATP hydrolysis.

References

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1P44

IF1 (mitochondrial FoF1 inhibitor protein); from single molecule to knock-out mouse
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Since a half century ago it has been known that IF1, an evolutionarily well-conserved mitochondrial protein, can inhibit the ATP hydrolysis activity of FoF1-ATP synthase (FoF1). Extensive in vitro biochemical works have established functional aspects of IF1 but little is known about how it modifies rotational catalysis of FoF1 and how it plays its physiological role in living cells and animals.

(1) We expressed human F1 in Escherichia coli cells and observed the rotation of single human F1 molecules. Driven by ATP hydrolysis, human F1 rotates very fast, over 1000 rps at 37°C, with pauses at new angular positions different from those of well-studied thermophilic F1. IF1 stops rotation irreversibly at the pausing angle of MgADP inhibition and the forced reverse rotation (to the direction of ATP synthesis) resumes rotation.

(2) IF1-knock-down human culture cells show normal glucose consumption, mitochondrial ATP synthesis and growth. Contrary to previous reports, the morphology of mitochondria in IF1-KD cells appears to be normal. When cells encounter sudden dissipation of pmf, the cytoplasmic ATP level in IF1-KD cells drops transiently while it remains unchanged in the control cells. When cells are exposed to reactive oxygen, IF1 alleviates cell injury.

(3) IF1-knock-out mouse was generated. Unexpectedly, the IF1-KO mice grow and breed normally. Response to starvation is also normal. No obvious changes in morphology of cells and of organelle including mitochondria are observed. We conclude that IF1 is not an essential protein for mouse. We are seeking the conditions under which IF1 plays a critical role.

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1P45

Visualization of ATP-driven Proton-Translocation of ATP Synthase Reconstituted in an Artificial Lipid Bilayer Membrane
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We succeeded in visualizing proton gradient formation via ATP synthase embedded in an artificial lipid bilayer membrane. The difference in the transmembrane ionic concentration, which is formed by many ion-transporting membrane proteins that exist on the cell membrane, is an essential energy source for life activity. However, the mechanism of ion pumping proteins is not well understood owing to the difficulties in handling membrane proteins and the limited methods for measuring their activity. We have developed a system to reconstitute an artificial lipid bilayer membrane under a microscope [1]. In this system, ATP synthase is reconstituted, and proton translocation activity is visualized as pH changes in the presence of ATP. For visualizing pH variation, pH-sensitive fluorescent dye fixed on a cover slip was used. Comparison of fluorescence intensity with and without ATP showed that the intensity was higher in the presence of ATP. Further, fluorescence intensity increased when ATP was added during observation. The reason for this observation may be that ATP synthase hydrolyzes ATP, transports protons between the lipid bilayer membrane and the glass plate, and then acidifies locally. In this result, we succeeded in visualizing proton pump activity of ATP synthase in vitro. In the future, we would like to visualize proton transportation at the single molecule level of ATP synthase to clarify the dynamics of proton transport.

Reference

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1P46

Physiological importance of the $\epsilon$ subunit of bacterial FoF1-ATP synthase
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Bacterial F0F1-ATP synthase is ubiquitous enzyme that synthesizes ATP using a proton motive force generated by the respiratory chain. Although this enzyme possesses ATP hydrolysis activity (reverse reaction) the primary function is generation of ATP. The $\epsilon$ subunit is