mechanism. Whereas these enzymes produce three ATP molecules per rotation in the F_1 triple stroke motor, the F_o motor transfers ions via turbines of variable size, the c rings. Structurally determined c ring sizes revealed coupling ratios (ions per ATP) between the F_o and F_1 motors geared from 3.3 to 5 depending on different species. We have measured the molecular mass of bacterial c rings by 'Laser induced liquid bead ion desorption' (LILBID). The novel method allows the mass determination of non-covalently assembled membrane protein complexes even in the MDa-range with high accuracy and therefore also allows the exact determination of the c ring stoichiometries and hence the enzyme's coupling ratios. It requires only microgram amounts of protein in detergent solution.

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S1. 45 Single pair FRET with fusion proteins of the F_0F_1-ATP synthases from *Escherichia coli*

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A rotor and a stator subunit of EF_0F_1 have previously been selectively labelled with appropriate organic fluorophores. Time resolved single pair fluorescence resonance energy transfer (spFRET) has revealed a stepwise rotational subunit movement during ATP synthesis (Diez et al., Nat. Struct. Mol. Biol. 2004). To simplify the labelling and reconstitution procedure necessary for double labelling of EF_0F_1 an enhanced green fluorescent protein (EGFP) was genetically fused to the γ-subunit. In order not to disturb the conformational changes during the catalytic steps, a leucine zipper helix was used as linker between the γ-subunit and EGFP. This helix elongated the C-terminus of the γ-subunit and its rotation was transduced to EGFP. The b-subunit contains the mutation b64C, which allows covalent labelling of the fusion protein with an organic acceptor fluorophore. This construct offers the opportunity to analyze the dynamics of the enzyme during ATP synthesis and ATP hydrolysis by spFRET with freely diffusing proteoliposomes. In addition fluorescence anisotropy measurements can be carried out with immobilised proteoliposomes.

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S1.46 Supramolecular organization of mitochondrial ATP synthases: Electron microscopy study

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Cryo-electron microscopy is applied to obtain a medium resolution structure of the dimeric ATP synthase in mitochondria. Although the enzyme functions as a monomer, dimeric ATP synthase supercomplexes were found in yeast, bovine heart, *Arabidopsis* and *Chlamydomonas*. Recently a very stable ATP synthase supercomplex was described in the alga *Polytomella*. The supercomplex includes a number of additional subunits that are speculated to be involved in dimer formation. Structural analysis by single particle analysis of negatively stained molecules revealed that monomers specifically interact via the F_0 parts and an angle between the two F_0 parts is about 70° in *Polytomella*. This arrangement is considered to induce a strong local bending of the membrane. In order to increase a resolution and to obtain a native state of the protein cryo-electron microscopy (EM) method was used. Preliminary EM data on a set of about 70,000 projections allow us to expect at least 20 Å resolution in a 3D model of the dimeric ATP synthase.

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S1. 47 Epsilon subunit, an ATP sensor of ATP synthase

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Our recent studies showed that the ε subunit of F_1-ATPase from the thermophilic *Bacillus* PS3 (TF_1) can bind ATP in a very specific manner. From these results, we have proposed a regulatory mechanism of ATP synthase involving ATP binding to the ε subunit. One of the critical issues is how the ATP binding to the ε subunit may concern with its regulatory role. To address this question, eleven mutants of the ε subunit were prepared, in which one of the basic or acidic residues was substituted with alanine to alter their ATP binding. ATP binding to these mutants was measured by gel-filtration chromatography. Among them, four mutants that showed no ATP binding were selected and subjected to further study. The mutant ε subunits can be reconstituted with the $\alpha_3\beta_3\gamma$ complex of TF_1. The ATPase activity of the resulting $\alpha_3\beta_3\gamma\varepsilon$ complexes was measured and the extent of inhibition by the mutant ε subunits was compared in each case. With one exception, weaker binding of ATP correlated with greater inhibition of ATPase activity. These results clearly indicate that ATP binding to the ε subunit plays a regulatory role and that ATP binding may stabilize the ATPase active form of TF_1 by fixing the ε subunit into the folded conformation.

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S1.48 Yeast cells depleted in subunit *h* fail to assemble subunit *6* within the ATP synthase and exhibit altered mitochondrial cristae morphology

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Within the yeast mitochondrial ATP synthase, subunit *h* is a nuclear-encoded protein belonging to the so-called "peripheral stalk". To examine the role of subunit *h* in ATP synthase function and assembly, we used a regulatable, doxycycline-repressible, subunit *h* gene, to overcome the strong instability of the mtDNA observed in deletion mutants. Yeast cells expressing less than 3% of subunit *h*, but still containing intact mitochondrial genomes, grew poorly on respiratory substrates because of a major impairment of ATP synthase-borne ATP synthesis, whereas the respiratory chain was not affected. The lack of ATP synthesis in the subunit *h*-depleted (d*h*) mitochondria was attributed to defects in the assembly/stability of the ATP synthase. A main feature of d*h* mitochondria was a very low content (<6%) in the mitochondrially encoded subunit *6*, a