The aim of the present study is to understand the mechanism of converting chemical energy into mechanical work in the rotary molecular motor F_oF_1-ATP synthase. Conformational changes of the catalytic subunit β which are assumed to drive rotation of the central stalk γ are the key to solve this question. In order to investigate them, we have applied single molecule TIRF microscopy with polarisation modulation to observation of $\alpha_3\beta_3\gamma$ subcomplex of F_oF_1-ATP synthase. Simultaneous but independent observation of conformational changes of β and rotational substeps of γ in single $\alpha_3\beta_3\gamma$ molecules revealed a sequence of conformational changes of the β subunit during ATP hydrolysis between "Open", "Closed", and "partially Closed" states. Consequently, it was revealed that the most crystal structures containing two nucleotide-bound β subunits represent the pre-hydrolysis state. Moreover, ATP-waiting state was found to correspond to a novel combination of conformation. Thus cooperative domain motions of three β subunits drive rotation of the central stalk subunit γ.

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S1.41 Single molecule analysis of the tentoxin-induced molecular processes of inhibition and stimulation of CF_1

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Tentoxin, a cyclic peptide produced by phytopathogenic fungi inhibits the chloroplast F_1-ATPase in sensitive plants at nanomolar to micromolar concentrations, whereas higher concentrations of tentoxin stimulate the activity up to 2–10 folds. By using the cyanobacterial F_1-ATPase obtained from *Thermosynechococcus elongates* BP-1 as a model enzyme, we analyzed both the inhibition and stimulation effects of tentoxin at a single molecule level, and successfully clarified the molecular steps involved in both processes. Inactivation delays the dwell time of a single step in the complete 360° turn of the γ subunit. Consequently the enzyme shows an asymmetric rotation. In contrast, rotation in stimulated F_1 particle is smooth and accompanied by strongly reduced ADP inhibition. Based on these observations and the kinetic analyses of the enzyme activity in the presence of tentoxin, the molecular processes of inhibition and stimulation of CF_1-type ATPase by tentoxin are discussed.

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S1.42 H^+-ATP synthase from chloroplasts: Rotational movement of subunits in single enzymes during ATP synthesis

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The ATPsynthase from chloroplasts forms ATP from ADP and phosphate. This energy-consuming reaction is driven by a transmembrane electrochemical potential difference of protons. Rotation of the γ subunit during ATP synthesis has been shown for *E. coli* ATPsynthase. Similar studies with a eukaryotic ATPsynthase during ATP synthesis have not been reported yet. In order to observe single enzymes we labelled the rotor-subunit γ at the cysteine C322 with ATTO532. An acceptor fluorophore, ATTO-655-AMPPNP, was introduced via substrate-enzyme-interaction at the so-called non-exchangeable ATP binding site. This procedure is necessary because cysteine mutants are not readily available for eukaryotic enzymes. Intermolecular FRET measurements of single double labelled enzymes reconstituted into liposomes were carried out with a home-built confocal microscope. During non-catalytic conditions we observed photon bursts with a constant FRET efficiency. During ATP synthesis bursts with changing FRET efficiencies were found indicating a distinct step-wise repeating sequence of FRET-levels (1-2-3-1-…). This corresponds to a 120° stepwise rotation of the γ-subunit relative to the non-exchangeable ATP binding site on the α-subunit.

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S1.43 Dimeric ATP synthase in thylakoid membranes of green algae and cyanobacteria

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To enlighten the supramolecular organisation of H^+-ATP synthases, native electrophoresis is the method of choice. Fundamental protein interactions are preserved during this kind of electrophoresis demonstrating the presence of stoichiometric supramolecular assemblies. While dimers and higher oligomers have been frequently described for the mitochondrial ATP synthase in biochemical and structural investigations, ATP synthase dimers in thylakoid membranes are much less characterised. In the chloroplasts of the green algae *Chlamydomonas reinhardtii*, we demonstrated the existence of such ATP synthase dimers. In contrast to the mitochondrial ones, the plastidic dimers dissociate upon addition of phosphate. The influence of this ATP synthase substrate on the supramolecular organisation suggested a physiological role of dimerisation. We could demonstrate that the dimer to monomer ratio of chloroplast ATP synthase is altered by the growth conditions. By labelling with stable isotopes, an increased assembly of ATP synthase dimers on the expense of pre-existing monomers during photomixotrophic growth compared to a photoautotrophic culture was observed. Our recent studies reveal the presence of ATP synthase dimers not only in *C. reinhardtii*, but also in thylakoid membranes of other alga and even in cyanobacteria. Our data demonstrate that dimers of chloroplast ATP synthases have an important physiological role and are common over a broad range of thylakoid containing organisms.

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S1.44 Mass determination of membrane protein complexes in detergent solution: The c rings from F-ATP synthases

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F_1F_o-ATP synthases are bipartite molecular motors able to convert an electrical membrane potential into ATP by a rotational

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