decarboxylation-driven Na+ pump use Na+ as the coupling ion. Specific binding sites for the respective coupling ion are present in the oligomeric ring of c subunits. In the c ring from *Ilyobacter tartaricus*, each sodium ion is coordinated by side chain and backbone oxygens from the inner and outer helices of two neighboring c subunits and the binding pocket is firmly stabilized by hydrogen bonds donated to the conserved cE65residue. In this conformation the sodium ion is buried and cannot reach putative ion conducting channels in subunit a. Cysteine–cysteine cross-linking studies between subunits a and c indicated subtle but distinct conformational changes around the Na+ binding site that were elicited by the stator arginine. These experiments support a model in which the alkali ion is released from the binding site by adapting a conformation that favors arginine but not Na+ binding. pH profiles for DCCD inhibitor binding by the proton translocating ATP synthase of *Halobacterium salinarium* reflected the popular group protonation mechanism of the conserved carboxylates in the c ring. However, all other ATP synthases investigated yielded pH profiles that could be best explained by the coordination of a hydronium ion. Hence, three different modes of ion binding (Na+, H3O+, H+) have developed in different ATP synthases during evolution.

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S1/2 Mechanistic insights of F_1-ATPase rotation from single-molecule measurements of the power stroke

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Single-molecule measurements of the rotation of the F_1-ATPase γ subunit from *E. coli* were made with a time resolution that allowed the power stroke (transition between dwells) to be resolved. The duration of transitions and dwells was measured as a function of the viscosity of the medium using PEG-400 with various sizes of gold nanorods attached to the γ subunit as a visible probe of rotation. In the absence of PEG, the power stroke was found to be viscous-limited when nanorods with dimensions of 91×45 nm were used as probes, but was kinetically-limited when 75×35 nm, 87×36 nm, and 90×46 nm were used. The dwell times matched ATPase turnover rates measured in bulk solution without an attached nanorod at low viscosities, and increased about 8 fold as a function of viscosity under conditions in which the power stroke was kinetically limited. When the power stroke became limited by the viscous drag, the dwell times became longer than the ATPase turnover time in the absence of a bound nanorod. Under these conditions, the increase in the transition time caused a 20 fold increase in the dwell time regardless of the size of the nanorod used. These results indicate that forcing the γ subunit to rotate more slowly than occurs by the intrinsic kinetically-limited mechanism causes the enzyme mechanism to deviate from its normal catalytic cycle, and provide insight into sequential conformational states of the enzyme during a catalytic cycle.

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S1/3 The stator stalk of *Escherichia coli* ATP synthase

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The peripheral stator stalk of *E. coli* ATP synthase is formed by a dimer of helical, 156-residue *b* subunits that are anchored in the membrane by N-terminal transmembrane segments. b_2 reaches up the side of F_1 and binds near the top, interacting with the δ subunit. Most of this distance is spanned by the dimerization domain contained within residues 53–122. Sequence analysis reveals an unusual, alanine-rich 11-residue repeat pattern, consistent with a novel, two-stranded right-handed coiled coil (RHCC) structure. Disulfide formation studies, and analysis of the shapes and stabilities of disulfide-linked forms, support an RHCC structure with helices offset by 5–6 residues, making the structure intrinsically asymmetric. The RHCC is controversial and a left-handed coiled coil has also been proposed. Chimeric forms of *b* incorporating exogenous sequences containing a hendecad pattern similar to that of *b* into the region between positions 55 and 95 supported ATP synthesis *in vivo*, but those incorporating known left-handed coiled coil sequences failed to do so, even though ATP synthase still assembled. Single residue deletions within the dimerization domain also support assembly but not ATP synthesis, confirming that the stator stalk has a functional role beyond simply holding on to F_1. The significance of the proposed offset RHCC structure in binding to F_1 and resisting rotational torque will be discussed.

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S1/4 Domain compliance and elastic power transmission in F_OF_1-ATPase

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F_OF_1-ATP synthase is composed from two stepping rotary motors coupled by a common rotary shaft. The electromotor, F_O, drives the chemical generator, F_1. Elastic power transmission between F_O is indispensible for their coupled operation at high speed. By fluctuation analysis the torsional spring constants of various enzyme domains were determined, engineered SS-bridges served to selectively stiffen others. Both, the central shaft in F_1 and, surprisingly, also the long eccentric bearing, were rather rigid. Only one domain of the rotor, namely where subunits γ and ε of F_1 contact the c-ring of F_O, was more flexible (50 pNnm) by order of magnitude. This elastic buffer, being located between the loci of torque delivery by F_O and by F_1, provides high kinetic efficiency to this twin engine under load, and it accounts for the ability of concerted action with different gears in different organisms.

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S1/5 Photoaffinity labeling and photoaffinity cross-linking of ATP synthase complexes

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Photoaffinity labeling and photoaffinity cross-linking have been proven to be valuable techniques for the localization and characterization of ligand binding sites. In order to characterize nucleotide binding sites of ATP synthases we have synthesized various mono- and bifunctional photoactivatable ATP analogs. The six nucleotide binding sites – three catalytic and three noncatalytic – of ATP synthases are

located on the F_1 complex of the enzyme (subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$) alternately at the interfaces between the major subunits α and β as demonstrated by photoaffinity labeling and photoaffinity cross-linking using mono- and bifunctional photolabels like 8-azido-ATP and 8,3′-diazido-ATP. In 1994 this interfacial location of all the nucleotide binding sites was confirmed impressively by X-ray analysis of the F_1ATPase from beef heart mitochondria by John Walker and coworkers. The introduction of an additional biotin residue, yielding 3′-biotinyl-8-azido-ATP, is advantageous for an easy detection of labeled proteins. Irradiation of F_1ATPases in the presence of 3′-biotinyl-8-azido-ATP resulted in the nucleotide-specific inactivation of the enzyme as well as in the nucleotide-dependent labeling of α and/or β subunits. Dimerization of 3′-biotinyl-8-azido-ADP resulted in the formation of the bifunctional diadenine dinucleotide 3′-dibiotinyl-8-diazido-AP_4A. Irradiation of F_1ATPases in the presence of 3′-dibiotinyl-8-diazido-AP_4A yielded the nucleotide-specific inactivation and the nucleotide-dependent formation of α–β cross-links. All these results demonstrate the suitability of the various azidonucleotides for photoaffinity labeling and photoaffinity cross-linking of ATP synthase complexes. In addition the AP_4A analogs should be very useful for the characterization of an adenylate kinase-like arrangement of nucleotide binding sites.

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S1/6 Structural organization of mitochondrial ATP synthase

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Specific modules and subcomplexes like F_1 and F_OF_O-parts, F_1-c subcomplexes, peripheral and central stalks, and the rotor part comprising a ring of c-subunits with attached subunits γ, δ, and ε can be identified in yeast and mammalian ATP synthase. Four subunits, $\alpha_3\beta_3$, OSCP, and h, seem to form a structural entity at the extramembranous rotor/stator interface ($\gamma/\alpha_3\beta_3$) to hold and stabilize the rotor in the holo-enzyme. The intramembranous rotor/stator interface (c-ring/a-subunit) must be dynamic to guarantee almost frictionless rotation. Unexpectedly, a c_{10}a-assembly could be isolated with almost quantitive yield suggesting that an intermediate step in the rotating mechanism was frozen under the conditions used. Isolation of dimeric a-subunit and $(c_{10})_2a_2$-complex from dimeric ATP synthase suggested that the a-subunit stabilizes the same monomer–monomer interface that had been shown to involve also subunits e, g, b, i, and h. The natural inhibitor protein Inh1 does not favor oligomerization of yeast ATP synthase. Other candidates for the oligomerization of dimeric ATP synthase building blocks are discussed, e.g. the transporters for inorganic phosphate and ADP/ATP that had been identified as constituents of ATP synthasomes. Independent approaches are presented that support previous reports on the existence of ATP synthasomes in the mitochondrial membrane.

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S1/7 Structure, function and regulation of the vacuolar ATPases

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The vacuolar (H^+)-ATPases (V-ATPases) are ATP-dependent proton pumps responsible for both acidification of intracellular compartments and proton transport across the plasma membrane. Intracellular V-ATPases function in membrane traffic processes, protein degradation, coupled transport of small molecules and the entry of various pathogens, including influenza virus. Plasma membrane V-ATPases function in renal acidification, bone resorption, pH homeostasis and tumor metastasis. The V-ATPases, which operate by a rotary mechanism, are composed of a peripheral domain (V_1) that hydrolyzes ATP and an integral domain (V_0) that conducts protons. These domains are connected by a central rotary stalk and peripheral "stator" stalks. Structural analysis using cysteine-mediated cross-linking and EM have allowed assignment of subunits to the central and peripheral stalks while analysis of gene fusions have suggested an ordered arrangement of subunits in the proteolipid ring of V_0. V-ATPase activity is regulated *in vivo* by reversible dissociation of the complex into free V_1 and V_0 domains, which are separately inactive. *In vivo* dissociation is a sensitive function of the cellular environment in which the V-ATPase resides. ATPase activity of the free V_1 domain is silenced by subunit H, which bridges the rotary and stator parts of the free V_1 domain, thus preventing rotation. We have recently begun to investigate the role of V-ATPases in tumor cell invasiveness.

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(S1) ATP synthase/ATPase symposium abstracts (poster and raised abstracts)

S1.8 Affinity purification of F-ATPases from mitochondria

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F-ATPases have been solubilized from bovine, ovine, porcine and yeast mitochondria with mild detergents. Their activities have been inhibited by residues 1–60 of the inhibitor protein, IF_1, from bovine mitochondria to which a six-histidine tag has been attached to the C-terminus. The inhibited complexes have been bound to a nickel-Sepharose column and, after washing steps, the pure enzyme-inhibitor complexes have been eluted in the presence of imidazole. Active enzyme has been released in a subsequent washing step. The active bovine enzyme has been reconstituted into phospholipid vesicles and its ability to synthesize ATP has been characterized. The subunit compositions of the various enzymes have been characterized by mass mapping of tryptic peptides. The core subunits of the enzyme that are required for catalysis are conserved in all the enzymes, and minor differences are found only in the compositions of the minor subunits. This single step purification yields active and well-coupled bovine enzyme. The purification of the F-ATPase from various species widens the scope of experiments to grow crystals of the intact enzyme complex.

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S1.9 ATP synthase as target of the tuberculosis antibiotic diarylquinoline

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