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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