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 $F_1$ATPase 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 $F_1$ATPases 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-$AP_4$A. Irradiation of $F_1$ATPases in the presence of 3′-dibiotinyl-8-diazido-$AP_4$A 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 $AP_4$A 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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Infection with *Mycobacterium tuberculosis* is a major world health problem leading to nearly 2 million deaths per year with about 2 billion people latently infected. Although antibiotics are available for tuberculosis (TB) treatment, multi-drug resistant strains as well as mycobacteria staying dormant within the host strongly demand development of drugs with new targets. Diarylquinolines (DARQs) are a novel class of antibiotics highly active against *M. tuberculosis*. In this study we validated ATP synthase as the antibiotic target using biochemical and binding assays. In a biochemical assay, DARQs inhibited ATP synthase at nanomolar concentrations. BIAcore studies with DARQ-coated chips revealed strong binding for purified ATP synthase, but not for the hydrophilic F1 part. Point mutations in hydrophobic subunit-c lead to decreased binding affinity. These results establish ATP synthase as the target for DARQs and suggest that inhibition of energy production is a promising approach for antibacterial drug discovery. Experiments to determine the effect of DARQs on mitochondria and to elucidate the role of ATP synthase in the physiologically dormant state of mycobacteria are under way, recent progress will be discussed.

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### S1.10 Keysteps during proton translocation by ATP synthases

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The proton motive force, consisting of the proton gradient ($\Delta pH$) and the electrical membrane potential ($\Delta\psi$), is the driving force of ATP synthesis in $H^+$-translocating enzymes. The two components are thermodynamically, but not kinetically equivalent. It is therefore speculated, that ATP synthases from different environments have adapted their ATP synthase to efficiently utilize the predominant driving force (high $\Delta\psi$ and low or inverse $\Delta pH$ in bacteria and mitochondria; low $\Delta\psi$ and high $\Delta pH$ in chloroplasts). We have compared the proton transport rates through the $F_0$ part of the ATP synthases of *E. coli*, spinach chloroplasts and the alkaliphilic bacterium *Bacillus* TA2.A1 in dependence of the proton concentration. We demonstrate, that enzymes from different origins show unequal pH profiles, if protons are pumped from the *P*-side to the *N*-side. We further introduced critical amino acid residues of the ATP synthase from the alkaliphilic bacterium in the enzyme of *E. coli* and obtained similar pH profiles as observed in the alkaliphilic wild type. Together with additional data, we propose a multistep model for $H^+$-transport through the $F_0$ part in hydrolysis and synthesis direction, in which the membrane potential and the proton gradient play distinct roles during $H^+$-translocation.

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### S1.11 Mutational analysis of the function of the βDELSEED-Loop of ATP synthase

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The DELSEED-Loop in the C-terminal domain of the β subunit of ATP synthase is often regarded as the structural element that drives rotation of γ during ATP hydrolysis. However, removal of all five negative charges in the DELSEED motif itself (→AALSAAA) had no significant effect on the rotational torque (Hara et al. (2000) JBC 275, 14260). A large portion of the interaction between the loop and γ is due to the conserved hydrophobic residues βI373, βI376, and βL377 (*E. coli* numbering). A recent molecular dynamics study implied specifically residues I376 and L377 of the $\beta_E$ subunit as important for driving rotation of γ (Pu and Karplus (2008) PNAS 105, 1192). We generated the single mutants βI373A, βI376A, βL377A, the double mutant βI376A/βL377A, and the triple mutant βI373A/βI376A/βI377A. Whereas the triple mutant failed to assemble, the other mutants were able to catalyze ATP synthesis and hydrolysis. Especially the double mutant showed activities that were slightly higher than wild-type. Interestingly, a βD372A/βI373A/βI376A/βL377A/βE381A quintuple mutant was assembled and showed reduced, but significant ATP synthesis and hydrolysis activity. The results indicate that more pronounced changes of the loop structure might be required to disrupt function. In our newest set of mutants, we are generating deletions of 3–4 amino acid residues.

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### S1.12 Supramolecular organization of the yeast $F_1F_o$-ATP synthase within the inner mitochondrial membrane and in detergent extracts

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The yeast mitochondrial $F_1F_o$-ATP synthase is a large complex of 600 kDa that uses the proton electrochemical gradient generated by the respiratory chain to catalyze ATP synthesis from ADP and Pi. For a large range of organisms, it has been shown that mitochondrial ATP synthase adopts oligomeric structures. Moreover, several studies have suggested that a link exists between ATP synthase and mitochondrial morphology. In order to understand the link between ATP synthase oligomerization and mitochondrial morphology, more information is needed on the supramolecular organization of this enzyme within the inner mitochondrial membrane. We have conducted an electron microscopy study on wild type yeast mitochondria at different levels of organization from spheroplast to purified ATP synthase complex. Using electron tomography, freeze fracture, negative staining and image processing we show that cristae form a network of lamellae, on which ATP synthase dimers assemble in linear and regular arrays of oligomers. Our results shed new light on the supramolecular organization of the $F_1F_o$-ATP synthase and its potential role in mitochondrial morphology.

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