physiology as they control ionic conditions in the cell and energise osmotic potentials, secondary transport schemes and ionotropic signalling. A surprising finding from the Na^+,K^+-ATPase structure was the docking of two conserved tyrosine residues at the C-terminus of the alpha subunit into the transmembrane domain, hinting that this was a previously unidentified regulatory element. Several mutations causing human neurological syndromes have subsequently been mapped to the C-terminal structure element, also clearly indicating that conservation of the structure is important for pump function. Mutational analysis confirmed this and prompted our further analysis by electrophysiology and molecular dynamics simulations, which have shown a profound effect of the C-terminus on the electrogenic transport properties. We further propose that the C-terminal region forms a binding pocket that can be exploited for pharmacological intervention in cardiovascular and neurological disease.

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Posters

2P.1 The role of the N-terminus domain of F_OF_1 inhibitory peptide from *Saccharomyces cerevisiae*: A kinetic approach

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In deenergized mitochondria, a small regulatory peptide called IF_1 (bIF_1 in bovine, yIF_1 in yeast) binds to ATP synthase and blocks ATP hydrolysis. In crystallized bovine F_1-ATPase in complex with IF_1, residues 22–49 of bIF1 are locked at an αβ catalytic interface, while residues 8–18 mainly interact with γ [1]. It was shown that deletion of residues 1–13 of bIF_1 did not affect its affinity for the enzyme [2], while deletion of residues 1–17 [2] or 1–21 [3] seriously decreased it. We have examined the consequences of length changes in the N-terminus part of yIF_1 on the kinetics of inhibition of isolated F_1-ATPase. Determination of the rate constant of IF_1 binding to F_1 (k_{on}) [4] and of its rate constant of dissociation from the complex (k_{off}) allowed to discriminate effects of yIF_1 modifications on binding site recognition and on the inhibited complex stability. Deletion of yIF_1 residues 1–13 (corresponding to bIF_1 residues 1–18) changed neither k_{on}, nor k_{off}. Deletion of residues 1–14 did not change k_{on} and slightly increased k_{off}. Deletion of residues 1–15 increased k_{on} by a factor 2 and k_{off} at least by a factor 20. We propose that residues 1–15 of yIF_1 do not play any role in molecular recognition and might even hamper it. On the other hand, residues 14–15 (but not residues 1–13) participate in the stabilization of F_1–IF_1 complex. Quite surprisingly, considerable lengthening of the N-terminus domain (by 44 residues) did not affect k_{on} despite the expected increase of steric hindrance. It increased k_{off} more than 10 fold. We conclude that IF_1 probably approaches the catalytic interface with its N-terminus tail folded back. Once IF_1 is bound by its medium domain to α and β subunits, the N-terminus spreads around the γ subunit. We are currently checking more in depth this model by attaching a small globular protein to the N-terminus domain of yIF_1. Our results also suggest that γ subunit, that interacts with the N-terminus part of IF_1, plays a minor role in the inhibition mechanism.

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2P.2 Mycobacterial ATP synthase as drug target

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Recently, ATP synthase has come into focus as a novel antibiotic target. Previously we showed that diarylquinolines, a new class of anti-tuberculosis compounds presently in phase III clinical tests, efficiently kill *Mycobacterium tuberculosis* by inhibiting ATP synthase [1, 2]. Diarylquinolines are highly selective, we found that the lead compound of this drug class inhibits mycobacterial ATP synthesis at nanomolar concentrations (IC_{50} < 10 nM), but has only a minimal effect on ATP synthesis in human mitochondria (IC_{50} > 200 μM) [3]. These results demonstrate that proteins of energy metabolism, although conserved among prokaryotes and eukaryotes, can nevertheless be used as efficient antibiotic targets. The molecular basis for the observed selectivity is presently under investigation in our laboratory. The affinity of ATP synthase for diarylquinolines decreased significantly in the presence of high salt concentrations, indicating that electrostatic interactions play an important role in binding of this inhibitor. However, competition experiments showed that diarylquinolines do not directly compete with protons for the same binding site. Pinpointing the binding niche of this drug is under way in and recent progress will be reported. As affinity of several ATP synthase inhibitors depends on this enzyme's mode of action (ATP synthesis/high PMF versus ATP hydrolysis/low PMF mode) we investigated the function of ATP synthase in two mycobacterial strains. Whereas inverted membrane vesicles were clearly active in ATP synthesis, they were unable to set-up a proton motive force with ATP. These results show that mycobacterial ATP synthase is strongly blocked in ATP hydrolysis mode. The physiological function of this enzyme appears to be synthesis of ATP, not maintenance of the proton motive force.

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2P.3 Comparison of high resolution structures of F_1 ATP synthase from mitochondria. Implications for the catalytic cycle of the enzyme

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More than 20 high resolution structures of mitochondrial F_1-ATPase have been obtained since the first structure was described in 1994 [1]. These structures have provided the basis for the description of the conformations of the three catalytic β-subunits in the ground state [2] and in a transition state intermediate during the catalytic cycle [3]. We have compared the crystal structures of F_1-ATPases from bovine and yeast mitochondria, obtained with crystals with various space groups and unit cell sizes. We have examined the way that F_1-complexes are packed in the crystal lattices to determine whether the

conformations of the various subunits in the F_1-complexes have been influenced by lattice contacts between F_1-complexes in the crystals. The conformations of the subunits in the $\alpha_3\beta_3$-domains are influenced little, if at all, by crystal contacts. Therefore, the interpretation of the conformations of these subunits as representing intermediates in the catalytic cycle is valid. Only when the crystals were highly dehydrated to decrease the dimensions of the unit cell, packing the F_1-complexes more closely in the crystal lattice, was any change apparent: there was a slight inward movement of the C-terminal helices of α-subunits. As has been noted many times in the past [3, 4], crystal contacts often influence the conformation of the central stalk significantly, and so the interpretation of the position that its exposed foot has adopted in the crystal structures, in relation to the rotary cycle of the central stalk, has to be carried out with caution.

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2P.4 Binding of the inhibitor proteins IF_1 to mitochondrial F_1-ATPases

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In the structures of the complexes of bovine F_1-ATPase with residues 1–60 of bovine $IF_{1\ [1]}$, and of the yeast F_1-ATPase with residues 1–53 of yeast $IF_{1\ [2]}$, a long α-helix in the inhibitor proteins is bound in a deep groove at a catalytic interface between the C-terminal domains of the β_{DP}- and α_{DP}-subunits. In order to assess the contributions of specific amino acids in bovine IF_1 to binding, point mutations have been introduced singly throughout the long α-helix, and the effects on inhibitory properties have been measured. These experiments show that bovine IF_1 is bound mainly *via* hydrophobic interactions between its long α-helix with the C-terminal domain of β_{DP}-subunit, and in one case with the β_{TP}-subunit. In addition, there is a significant salt bridge between residue E30 in the inhibitor and residue R408 in the β_{DP}-subunit. Yeast IF_1 is bound in a similar way, but in the long α-helix there are significant local differences. The inhibitors also differ in the way that their N-terminal regions bind to F_1-ATPase. Residues 14–18 of bovine IF_1 form a short α-helix that interacts with the γ-subunit in the central stalk of the enzyme, whereas the equivalent region of yeast IF_1 has an extended loop structure that forms a salt bridge network with the γ- and α_E-subunits. Bovine IF_1 is a more potent inhibitor than yeast IF_1. The K_i values are: bovine IF_1 1–60 with F_1-ATPase 29.8 nM^{-1}, and with yeast F_1-ATPase 7.1 nM^{-1}; yeast IF_1 (E21A) with yeast F_1-ATPase, 16.0 nM^{-1}, and with bovine F_1-ATPase 217.5 nM^{-1}.

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2P.5 Combination of single molecule FRET spectroscopy with optical tweezers: A powerful tool for mechanistic studies of enzymes

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The H^+-ATP synthase forms ATP, the energy currency of the cell, from ADP and phosphate. This energy-consuming reaction is driven by a transmembrane electrochemical potential difference of protons. The H^+-ATP synthase has been labelled by two fluorophores which allow fluorescence resonance energy transfer (FRET). The FRET efficiency strongly depends on the distance between the fluorophores. This effect can be used to measure distances and changes in distances between the labelled subunits of the protein. The H^+-ATP synthase is reconstituted into liposomes and fluorescence bursts are observed when a single proteoliposomes traverses the detection volume of the confocal microscope. During the burst (duration 100 ms on average) FRET and FRET changes can be observed. This time is often too short to observe a full catalytic cycle. To increase the detection time we trap a single proteoliposome with an optical trap exactly in the centre of the confocal detection volume, so the duration of the burst is not controlled by diffusion of the proteoliposome. By this approach we obtain longer observation times, which allow a detailed analysis of intramolecular movements of subunits. With this combination of optical tweezers and single molecule fluorescence it is possible to investigate the mechanism of membrane integrated or associated proteins in a nature-like environment in long-time studies and the problem arising from immobilisation of the enzyme is avoided.

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2P.6 Single molecule spectroscopy of membrane bound H^+-ATP synthases from chloroplasts

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Subunit movements within the H^+-ATP synthase from chloroplasts ($CFOF_1$) are investigated by single molecule spectroscopy during ATP synthesis. The γ-subunit is covalently labeled at the disulfide bond between γC199 and γC205 with a fluorescence donor (ATTO532). A fluorescence acceptor (ATP-ATTO665) is non-covalently bound to a non-catalytic site at one α-subunit. The donor and acceptor labeled $CFOF_1$ is integrated into the liposomes and a transmembrane pH-difference is generated by an acid base transition. Single-pair fluorescence resonance energy transfer is measured in freely diffusing proteoliposomes with a confocal two channel microscope. The fluorescence time traces reveal a repetitive three stepped rotation of the γ-subunit relative to the α-subunit during ATP synthesis. During catalysis the central stalk interacts, with equal probability, with each αβ-pair. Without catalysis the central stalk interacts with only one specific αβ-pair and no stepping between FRET levels is observed.

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