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Abstracts

S2 ATP SYNTHASE/ATPase

Lectures

2L.1 Structural basis of ion binding modes in the F_O rotor of H^+ and Na^+ translocating ATP synthases

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A membrane-embedded rotor ring of hairpin-like c-subunits plays a central role in ion translocation during adenosine triphosphate (ATP) synthesis by proton- or sodium motive force-dependent F_1F_O-ATP synthases. The careful design of c-ring rotors in different species defines both rotor size and ion binding specificity, two important parameters in cell bioenergetics. It appears that the structure of these rotor rings represents a finely-tuned adaptation of the enzyme to the environment of the corresponding organism. To our knowledge today, the stoichiometry of rotor rings is constant within a species but variable (from 10 to 15 hairpins are known) among different species. In the Na^+-binding c_{11} ring of *Ilyobacter tartaricus* and the H^+-binding c_{15} ring from *Spirulina platensis*, the translocated ions are bound within the groove of two adjacent c-subunits in a coordination network including a conserved glutamate (or aspartate). Both structures suggest that the precise coordination chemistry keeps the ion (H^+ and Na^+) in an ion locked conformation during the passage through the lipid/c-ring interface. The notion is supported by combined structural, biochemical and also *in silico* generated data of the proton binding site. Exchange of the ion, including subtle conformational adaptations of the ion binding glutamate, would exclusively occur in a more hydrophilic environment, such as it presumably is the case at the rotor–stator interaction site, the a-subunit/c-ring interface. Furthermore, it now becomes structurally evident that a new type of ion coordination needs to be considered in the operation mode of some of the F_O motors.

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2L.2 ATP hydrolysis in ATP synthases can be differently coupled to proton transport and modulated by ADP and phosphate: A structure based model of the mechanism

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In the ATP synthases of *Escherichia coli* ADP and phosphate exert an apparent regulatory role on the efficiency of proton transport coupled to the hydrolysis of ATP. Both molecules induce clearly biphasic effects on hydrolysis and proton transfer. At intermediate concentrations (about 0.5–1 μM and higher) ADP inhibits hydrolysis and proton transfer; a quantitative analysis of the fluxes however proves that the coupling efficiency remains constant in this concentration range. On the other hand at nanomolar concentrations of ADP (a level obtainable only using an enzymatic ATP regenerating system) the efficiency of proton transport drops progressively, while the rate of hydrolysis remains high. Phosphate, at concentrations 0.1 mM, inhibits hydrolysis only if ADP is present at sufficiently high concentrations, keeping the coupling efficiency constant. At lower ADP levels phosphate is, however, necessary for an efficiently coupled catalytic cycle. We present a model for a catalytic cycle of ATP hydrolysis uncoupled from the transport of protons. The model is based on the available structures of bovine and yeast F_1 and on the known binding affinities for ADP and P_i of the catalytic sites in their different functional states. The binding site related to the inhibitory effects of P_i (in association with ADP) is identified as the $\alpha_{HC}\beta_{HC}$ site, the pre-release site for the hydrolysis products. We suggest, moreover, that the high affinity site, associated with the operation of an efficient proton transport, could coincide with a conformational state intermediate between the $\alpha_{TP}\beta_{TP}$ and the $\alpha_{DP}\beta_{DP}$ (similar to the transition state of the hydrolysis/synthesis reaction) that does not strongly bind the ligands and can exchange them rather freely with the external medium. The emptying of this site can lead to an unproductive hydrolysis cycle that occurs without a net rotation of the central stalk and, consequently, does not translocate protons.

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2L.3 Ion transport by the sodium pump

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The first crystal structure of the Na^+,K^+-ATPase revealed the potassium-bound form of the pig kidney enzyme at 3.5 Å resolution. This large membrane protein complex consists of an alpha subunit similar to the Ca^{2+}-ATPase, a heavily glycosylated beta subunit and a small regulatory gamma subunit (also known as FXYD2). The electrogenic transport performed by the Na^+,K^+-ATPase causes extrusion of three sodium ions and uptake of two potassium ions per ATP split. The gradients thus formed are of fundamental importance in

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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