ATP synthase from human p^0 (rho zero) cells was almost fully assembled in spite of the absence of subunits a and A6L using clear native electrophoresis (CNE or CN-PAGE). From this we conclude that subunits a and A6L are the last subunits to complete the ATP synthase assembly. Under the CNE conditions small amounts of dimeric and even tetrameric forms of the large assembly intermediate were preserved, suggesting that it associated further into higher order structures in the mitochondrial membrane. This result was comparable to the reduced amounts of dimeric and tetrameric ATP synthases from yeast subunit e and g null mutants detected by CNE. The dimer/oligomer-stabilizing effects of subunits e/g and a/A6L seem additive in human and yeast cells. The mature IF_1 inhibitor was specifically bound to the dimeric/oligomeric forms of ATP synthase and not to the monomer whereas nonprocessed pre-IF_1 still containing the mitochondrial targeting sequence was selectively bound to the monomeric assembly intermediate in p^0 cells and not to the dimeric form. This supports previous suggestions that IF_1 plays an important role in the dimerization/oligomerization of mammalian ATP synthase and in the regulation of mitochondrial structure and function.

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2P.39 Resolving stepping rotation of V-ATPase with an essentially drag-free probe

Shou Furuike[1,2], Masahiro Nakano[3,4], Kengo Adachi[1], Hiroyuki Noji[4], Kazuhiko Kinosita Jr.[1], Ken Yokoyama[3,5]
[1]*Department of Physics, Faculty of Science and Engineering, Waseda University, Japan*
[2]*Department of physics, Osaka medical college, Japan*
[3]*Chemical Resources Laboratory, Tokyo Institute of Technology, Japan*
[4]*Institute of Scientific and Industrial Research, Osaka University, Japan*
[5]*ICORP, ATP Synthesis Regulation Project, Japan Science and Technology Agency (JST), Japan*
E-mail: yokoyama.k.ab@m.titech.ac.jp

Vacuole-type ATPases (V- or VoV1-ATPases), together with F_OF_1 ATP synthases, constitute a superfamily of rotary molecular machines that couple ATP hydrolysis/synthesis in the soluble V_1/F_1 portion with proton (or Na^+) flow in the membrane-embedded V_O/F_O portion through rotation of a common central shaft. Here we have observed at submillisecond resolutions the ATP-driven rotation of isolated V_1 and of the whole V_OV_1 from *Thermus thermophilus*, by attaching a 40-nm gold bead for which viscous drag is almost negligible. At saturating ATP of 4 mM, V_1 rotated at about 60 revolutions/s, with about 5 ms dwells every 120°. Dwell time analyses indicated that at least two events other than ATP binding, one likely ATP hydrolysis, occur in each dwell, as in F_1. Unlike F_1, however, the dwells were at ATP-waiting positions that were resolved at μM ATP. V_OV_1 rotated an order of magnitude slower, and exhibited dwells separated by about 30°. The twelve positions, though not always fully populated, match the twelve-fold symmetry of the V_O rotor in *T. thermophilus*, indicating that the ATP-driven rotation must go through stator–rotor interactions in V_O.

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2P.40 Heterologous expression of the peripheral stalk *Aquifex aeolicus* F_1F_O ATP synthase in *Escherichia coli*

Chunli Zhang, Guohong Peng, Hartmut Michel
Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, Frankfurt am Main, Germany
E-mail: Hartmut.Michel@biophys.mpg.de

The hyperthermophilic bacterium *Aquifex aeolicus* possesses a nine-subunit F_1F_O ATP synthase [1]. A part of the complex, called the peripheral stalk, provides the connection between the membrane embedded F_O part and the soluble F_1 part, acting as a stator to counteract the rotation of the catalytic F_1 part during ATP synthesis. Structural information is available to date for the peripheral stalk subunits of the bovine mitochondrial F_1F_O ATP synthase and the *Thermus thermophilus* A_1A_O ATP synthase, respectively [2–5]. However, further structural characterization is necessary because the peripheral stalk is the least conserved component of the complex, differing substantially in composition and stoichiometry among ATP synthase subtypes [5]. In particular, in *A. aeolicus*, the peripheral stalk is exceptional because it is hetero- and not homodimeric and so it differs from that of all other currently known F_1F_O ATP synthases of non-photosynthetic organisms [1]. It mainly contains subunits b_1 and b_2, encoded by genes *aq_1586* and *aq_1587*, which overlap by 1 bp in the genome. We have cloned the two genes and expressed the b_1/b_2 subunits heterologously in *Escherichia coli*. They localize both in *E. coli* membranes and inclusion bodies. Two-dimensional Blue native (2-D BN)/SDS-PAGE, together with peptide mass fingerprint mass spectrometry (PMF-MS) shows that they form a complex in *E. coli* membranes. The b_1/b_2 complex can be isolated from the membranes to a high level of purity in a single chromatographic step. Further studies are in progress to optimize the expression level and to characterize the folding and stability of the b_1/b_2 complex by size exclusion chromatography, circular dichroism and differential scanning calorimetry. The final aim of the project is the determination of the structure of the b_1/b_2 complex by 3-D crystallography.

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