the role of the P-loop residue Ser238 in phosphate-binding. The structures display novel conformations in the P-loop which are believed to represent important intermediates on the catalytic pathway. Comparison of the wild type structure of subunit A with the mutant S238A reflects its central role in the unique arched P-loop structure of A in A-ATP synthases and suggests an important evolutionary switch in P-loop and thereby in nucleotide recognition and mechanism of ATP synthesis and/or ATP hydrolysis of the biological machines A-, F-ATP synthases and V-ATPase.

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2P.19 Biochemical and structural investigations of the *Ilyobacter tartaricus* F_O ATP synthase

Jonna Hakulinen[1], Jan Hoffmann[2], Luise Eckhardt-Strelau[1], Bernd Brutschy[2], Thomas Meier[1]
[1]*Department of Structural Biology, Max-Planck-Institute of Biophysics, Max-von-Laue Str. 3, 60438 Frankfurt/Main, Germany*
[2]*Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt/Main, Germany*
E-mail: jonna.hakulinen@biophys.mpg.de

Adenosine triphosphate (ATP) synthase catalyzes the synthesis of ATP from ADP and phosphate by the dissipation of a transmembrane electrochemical ion gradient, which can be created by the respiratory chain complexes. The enzyme consists of two main subcomplexes F_1 and F_O that both function as the rotary motors. The water-soluble F_1 consists of subunits $\alpha_3\beta_3\gamma\delta\varepsilon$ and harbours the three nucleotide catalytic binding-sites. In bacteria the membrane-embedded F_O subcomplex consists of a ring of 10–15 c-subunits, which rotates against the neighbouring stator a- and b_2-subunits, thereby conducting ions across the membrane. Details for the ion translocation and mechanism and torque generation in the F_O motor are available on the basis of biochemical data and structures of the c-ring but structural data on the a-subunit is completely missing. In the bacterium *Ilyobacter tartaricus*, a-subunit is a hydrophobic protein of about 32 kDa size, consisting of five or six transmembrane α-helices. It is proposed to be part of the water-accessible access pathways to and from the rotor ion binding sites and to provide a key arginine, which forms reversible contacts with glutamates on the c-subunits of the rotor ring during the ion translocation. The aim of this work is the biochemical and structural characterization of the F_O subcomplex from *I. tartaricus* F_1F_O-ATP synthase. The whole enzyme was heterologously expressed in *Escherichia coli* host cells. Either the whole enzyme or the F_O subcomplex, after separation from F_1, was purified by affinity chromatography from the solubilized membrane fraction. Size-exclusion chromatography and Blue Native polyacrylamide gel electrophoresis confirm that both complexes (F_1F_O and F_O) are intact and fully assembled. The correct mass and subunit composition of the holo-enzyme (F_1F_O) and of the isolated F_O-subcomplex was furthermore determined and confirmed by laser-induced liquid bead ion desorption mass spectrometry (LILBID-MS). The purified F_O-subcomplex was successfully reconstituted into lipid vesicles and first structural investigations by electron microscopy are presented.

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2P.20 Down-regulation of F_1 ε subunit in HEK293 cells

V. Havlíčková, V. Kaplanová, H. Nůsková, Z. Drahota, L. Stibůrek, J. Houštěk
Department of Bioenergetics, Institute of Physiology and Center of Applied Genomics, Academy of Sciences of the Czech Republic
E-mail: vendula.havlickova@post.cz

The mammalian subunit ε is the smallest and functionally less characterized subunit of F_1 catalytic part of ATP synthase. The mammalian subunit ε encoded by *ATP5E* gene is a 5.8 kDa protein that lacks a cleavable import sequence. Compared to other F_1 subunits, ε is the only one without a homolog in bacteria or chloroplasts. Complementation studies confirmed that the yeast and mammalian ε are structurally and functionally equivalent [1]. F_1 subunits γ, δ and ε together with c-subunits oligomer form the rotor of ATP synthase [2]. Disruption of the *ATP15* gene encoding ε subunit in yeast resulted in no detectable oligomycin-sensitive activity, decreased content of γ, δ and F_O subunits and in F_1 instability [3]. It was also associated with accumulation of a/b dimer [4]. Here we report that silencing of ATP5E gene leads to a decrease of activity and protein content of mitochondrial ATP synthase complex and ADP-stimulated respiration in mammalian HEK293 cell to approximately 40% of the control. Decreased amount of ε subunit in *ATP5E* silenced cell lines was accompanied by a decreased content of the F_1 subunits α and β and as well as the F_O a- and d-subunits, while the content of F_O c-subunit was not affected. We found the accumulated c-subunit to be present in fully assembled ATP synthase complex and in subcomplexes of 200–400 kDa, which contained neither F_1 subunits α and β, nor the F_O subunits a, b or d. Our study shows that ε subunit is necessary for assembly and/or stability of the F_1 catalytic part of the mammalian ATP synthase and it is also important for incorporation of the hydrophobic subunit c into F_1-c oligomer during ATP synthase biogenesis.

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2P.21 Adaptations of the ATP synthase a-subunit to support synthesis at low protonmotive force at both pH 7.5 and 10.5 may underpin the more stringent requirement for lysine-180 in TMH-4 by alkaliphilic *Bacillus pseudofirmus* OF4 than by more modestly alkaliphilic thermoalkaliphile *Bacillus* sp. TA2.A1

Makoto Fujisawa, Jun Liu, Oliver Fackelmayer, Terry A. Krulwich, David B. Hicks
Mount Sinai School of Medicine, Dept. of Pharmacology and Systems Therapeutics, USA
E-mail: david.hicks@mssm.edu