References
[1] Bowler M. W, Montgomery MG, Leslie AGW, Walker JE (2007) *J. Biol. Chem.* **282:** 14238–14242.
[2] Kabaleeswaran V, Puri N, Walker JE, Leslie AGW, Mueller DM (2006) *EMBO J.* **25:** 5433–5442.
[3] Stocker A, Keis S, Vonck J, Cook GM, Dimroth P (2007) *Structure* **15:** 904–914.
[4] Yagi H, Kajiwara N, Tanaka H, Tsukihara T, Kato-Yamada Y, Yoshida M, Akutsu H (2007) *Proc. Natl. Acad. Sci. U. S. A.* **104:** 11233–11238.

doi:10.1016/j.bbabio.2010.04.109

2P.13 ATP synthesis by the isolated and reconstituted monomeric mitochondrial H^+-ATP synthase from yeast

Kathrin Förster[1], Paola Turina[2], Friedel Drepper[3], Wolfgang Haehnel[3], Susanne Fischer[1], Peter Gräber[1], Jan Petersen[1]
[1]*Institut für Physikalische Chemie, Albert-Ludwigs Universität Freiburg, Germany*
[2]*Dipartimento di Biologia, Università di Bologna, Italy*
[3]*Lehrstuhl für Biochemie der Pflanzen, Institut für Biologie II, Albert-Ludwigs Universität Freiburg, Germany*
E-mail: kathrinfoerster@gmx.de

The H^+/ATP synthase from yeast mitochondria, MF_OF_1, was isolated, purified and reconstituted into liposomes prepared from phosphatidylcholine and phosphatidic acid. Analysis by mass spectrometry revealed the presence of all known subunits of the yeast enzyme ($\alpha_3\beta_3\gamma\delta\varepsilon$ 45ad89$_{10}$HfJge) with the exception of the K-subunit. The MF_OF_1 liposomes were energized by acid base transitions and K^+/valinomycin diffusion potentials and high rates of ATP synthesis are observed. Titration of the number of MF_OF_1 per liposome indicates that the monomeric enzyme is able to catalyze high rates of ATP synthesis similar to that observed under physiological conditions. ATP synthesis was abolished by addition of uncouplers, as well as by the specific inhibitor oligomycin. The rate of ATP synthesis was measured as a function of pH_{out}, pH_{in} and the phosphate concentration. Maximal rates (turnover number) of approx. 100 s^{-1} are observed at a transmembrane pH difference of 3.2 U (at $pH_{in} = 4.8$ and $pH_{out} = 8.0$), in the presence of a superimposed transmembrane electric potential difference of 133 mV (Nernst potential). The apparent K_M for P_i depends on the pH_{out}.

doi:10.1016/j.bbabio.2010.04.110

2P.14 IF_1 influences on energy metabolism not only under energy restricted condition but also under normal condition

Makoto Fujikawa[1], Masako Mori[1], Masasuke Yoshida[1,2]
[1]*JST, ICORP, Japan*
[2]*Kyoto Sangyo University, Faculty of Engineering, Japan*
E-mail: fujikawa@atp.miraikan.jst.go.jp

IF_1 is a mitochondrial protein coded in nuclear genome [1, 2]. It is established that IF_1 binds mitochondrial F_OF_1-ATP synthase and inhibits its ATP hydrolysis activity. On the other hand, IF_1 does not influence ATP synthesis reaction. According to such properties, IF_1 is assumed to play a role in suppression of wasteful consumption of ATP by inhibiting the reverse reaction of F_OF_1-ATP synthase. However, physiological function of IF_1 remains poorly understood. We, here, studied it by using HeLa cells whose IF_1 expression is knocked down by short hairpin RNA (IF_1-KD cells). In the IF_1-KD cells, the ATP level decreased faster than that in the mock-treated cells under chemical ischemia as previously reported [3]. We show that IF_1 influences on energy metabolism under normal condition; the metabolic rate of IF_1-KD cells was higher than that of the mock cells under normal medium cultivation to compensate wasteful hydrolysis of ATP by F_OF_1-ATP synthase. In addition, we will report that IF_1 has a wide and long-range effect on energy metabolism.

References
[1] Ichikawa N *et al.* (1999) *Biosci. Biotechnol. Biochem.* **63:** 2225–2227.
[2] Yamada E *et al.* (1997) *Immunology* **92:** 571–576.
[3] Campanella M *et al.* (2008) *Cell Metab.* **8:** 13–25.

doi:10.1016/j.bbabio.2010.04.111

2P.15 A possible role for the shift between inner and outer membranes in the rotor ring of F- and V-ATPases

Christoph Gerle
Career Path Promotion Unit for Young Life Scientists, Kyoto University, Japan
E-mail: cgerle@cp.kyoto-u.ac.jp

F- and V-ATPases fulfil their energy transforming function by a rotary mechanism. A tenet of rotary catalysis by F- and V-ATPases is that their transmembrane stator stays tightly connected to the adjacent rotor ring during rotation. It is still not clear how the rotor and stator complex interact in order to stabilize the rotary complex even during a relative rotation of the two domains with a frequency up to 500 Hz [1]. A remarkable feature of their proton or sodium transporting rotor ring is that they contain an inner lipid membrane. An even more remarkable feature is a relative shift of the inner lipid membrane towards the periplasmic side. This shift between inner and outer membranes was observed in high resolution structures of rotor rings from evolutionary distant rotary ATPases and thus appears to be a general feature [2–4]. According to the half-channel model of torque generation in F_O/V_O rotary motors, a water filled half-channel permits access to ion binding sites of the rotor ring from the periplasmic side. The ion-binding sites are situated close to the center of the outer membrane hydrophobic core. Therefore, the difference in height of the outer and inner membranes brings the periplasmic half-channel in close proximity to the water filled cytoplasmic inside of the rotor ring. The electric potential between the periplasmic and cytoplasmic sides is expected to fall-off where the insulation is thinnest. I speculate that the shift between the inner and outer membranes in rotor rings results in a close proximity of the water filled periplasmic half-channel and bulk water of the cytoplasmic inside of the rotor ring. The expected distance of less than 12 Å is far shorter than the diameter of a typical membrane. This should lead to a radial potential fall-off between the stator and rotor ring. The resulting electric field between the stator and rotor ring could stabilize the complex during rotation. Importantly, an increased rotational speed due to a higher transmembrane potential would be accompanied by a stronger attraction.

References
[1] von Ballmoos *et al.* (2009) *Annu. Rev. Biochem.* **78:** 649–672.
[2] Pogoryelov *et al.* (2009) *Nat. Struct. Mol. Biol.* **16:** 1068–1073.
[3] Meier *et al.* (2005) *Science* **308:** 659–662.
[4] Murata *et al.* (2005) *Science* **308:** 654–659.

doi:10.1016/j.bbabio.2010.04.112