membrane damage) or are we dealing with a new proton transporter was open. In this work, TCP-C15 was shown to slowly incorporate into membrane. Therefore, by long incubation (10–20 min) we showed an authentic uncoupling effect (stimulation of mitochondrial respiration) with nanomolar concentrations of this compound. With the help of a new experimental method, it was shown that the mitotropic compound of SkQ set, which in reductive conditions in the mitochondrion transforms into the hydroquinone form, shows uncoupling effect at nanomolar concentrations.

References

[1] Motovilov K.A. *et al.* (2009) *Biochemistry (Moscow) Supplemental Series A: Membrane and Cell Biology* **3:** 478–487.
[2] Antonenko Y.N. *et al.* (1993) *Biochim. Biophys. Acta* **1150:** 45–50.
[3] Yaguzhinsky L.S. *et al.* (2006) *Biochim. Biophys. Acta* **1757:** 408–414.

doi:10.1016/j.bbabio.2010.04.147

3P.5 Establishing the role of the mitochondrial carrier MTCH2

Atan Gross*[,1,2], Alan J. Robinson[2], Edmund R.S. Kunji[2]
[1]*Department of Biological Regulation, The Weizmann Institute of Science, Rehovot 76100, Israel*
[2]*Medical Research Council, Mitochondrial Biology Unit, Hills Road, Cambridge CB2 0XY, United Kingdom*
E-mail: atan.gross@weizmann.ac.il

Mitochondrial outer-membrane permeabilization (MOMP), leading to cytochrome *c* release and caspase protease activation is a major event during apoptosis. BCL-2 family members are pivotal regulators of MOMP, but it is unknown how they regulate this process. BID, a pro-apoptotic BCL-2 family member, plays a critical role in executing apoptosis in the liver by initiating MOMP. Previously, mitochondrial carrier homolog 2 (MTCH2) was demonstrated to interact with BID in cells signaled to die. MTCH2 is a novel and previously uncharacterized 33-kDa protein, which belongs to the mitochondrial carrier family. By using a conditional knockout mouse MTCH2 was demonstrated to be essential for BID-induced apoptosis in the liver, but its mechanism of action remains unknown. A detailed bioinformatic analysis was carried out to assess how closely related MTCH2 is to other mitochondrial carriers. MTCH2 has the same structural features and topology as other mitochondrial carriers, but it has a unique substrate binding site, suggesting that the putative substrates differ chemically and structurally from known carrier substrates. In addition, MTCH2 has incomplete matrix and cytoplasmic salt bridge networks, indicating a low energy barrier to conformational changes. How will the substrates of MTCH2 be identified? The observation that MTCH2 has a unique substrate binding site with well-defined chemical features may help to narrow down substrate candidates. In addition, MTCH2 orthologs do not exist in yeast and plants, indicating that the substrate either does not exist or does not need to be transported into mitochondria in these organisms. To screen for substrate candidates the human MTCH2 was expressed in the cytoplasmic membrane of the Gram-positive bacterium *Lactococcus lactis*. The main advantage of using this system is that transport assays for the identification of the substrate can be performed with whole cells, as the carrier is directly accessible to the provided substrates. Establishing the role of MTCH2 as a mitochondrial carrier may have important implications for mitochondrial metabolism and the regulation of MOMP by BCL-2 family members during apoptosis.

*Visiting Scientist at (2).

doi:10.1016/j.bbabio.2010.04.148

3P.6 Identification and characterization of functional residues in a multi-subunit type Na^+/H^+ antiporter Mrp complex from alkaliphilic *Bacillus pseudofirmus* OF4

Masato Morino[1], Shinsuke Natsui[1], Tomohiro Ono[2], Talia H. Swartz[3], Terry A. Krulwich[3], Masahiro Ito[1,2]
[1]*Graduate School of Life Sciences, Toyo University, Oura-gun, Gunma 374-0193 Japan*
[2]*Faculty of Life Sciences, Toyo University, Oura-gun, Gunma 374-0193 Japan*
[3]*Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, New York 10029, USA*
E-mail: ito1107@toyonet.toyo.ac.jp

Mrp antiporters catalyze secondary $Na^+(Li^+)/H^+$ antiport and/or K^+/H^+ antiport that is physiologically important in diverse bacteria. Mrp is unique among antiporters in requiring all six or seven hydrophobic gene products (MrpA to MrpG) of the *mrp* operon for antiport activity. The MrpA, D and C proteins of the MrpA-B-C-D module have sequence similarity with Complex I subunits whereas the MrpE-F-G module is Mrp-specific [1,2]. A panel of site-direct mutants in 28 conserved or specific motif-related amino acid residues from an alkaliphile Mrp Na^+/H^+ antiporter was constructed. Each mutant transporter was expressed in antiporter-deficient *Escherichia coli* strain KNabc and the mutations were classified into 7 groups based on assays of growth/sodium-resistance, antiport properties, Mrp subunit levels, and formation of monomeric and dimeric Mrp complexes that are the active forms [1]. The analyses identified charged residues that are important for antiport activity and that are conserved across the large Mrp subunits of the MrpA-D module, MrpA, MrpD, as well as membrane-bound subunits (NuoL/M/N) of complex I. These included MrpA-K223, -K299 and MrpD-K219 as well as acidic residues that had been identified in *Bacillus subtilis* Mrp [3]. This study also extended evidence for a key role for MrpE of the MrpE-F-G module. MrpE is required for normal membrane levels of other Mrp proteins and normal complex formation. Conversely, some mutations in the MrpA-to-D module affected membrane levels of MrpE. Residues that are required for formation of the monomeric form or both forms of hetero-oligomeric Mrp complexes were identified for the first time. A mutation of Proline81 in MrpG produced a novel Mrp that supported sodium-resistance but lacked antiport activity. While a pair of tyrosines and a VFF motif with proposed roles in sodium-binding were mutated without effect, mutation of MrpA-H700 of a putative quinone binding site affected K_m values for the activity.

References

[1] Morino *et al* (2008) *J. Bacteriol.* **190:** 4162–4172.
[2] Mathiesen, Hagerhall (2003) *FEBS Lett.* **54:** 7–13.
[3] Kajiyaama *et al.* (2009) *Microbiology* **155:** 2137–2147.

doi:10.1016/j.bbabio.2010.04.149

3P.7 Effect of cardiolipin on the iron uptake of F_1F_OATP synthase in heart mitochondria

Misun Kim, Eunsook Song
Department of Biological Science, Sookmyung Women's University, South Korea
E-mail: eunsong@sm.ac.kr

Excessive iron is known to amplify ROS by Fenton and Haber–Weiss reaction, with subsequent damage of proteins, lipids and DNA of mitochondria. As one of the components affected by iron, cardiolipin, a tetra-acyl phospholipid, is crucial for oxidative phosphorylation of mitochondria. Recently, we showed that iron uptake was