We focused on extracellular flagellar filament assembly and complementation of motility in *B. subtilis* flagellin mutant. First, we constructed the flagellin defect strain of *B. subtilis* using the chromosomal homologous recombination. Complementation test was carried out with *B. subtilis* flagellin mutant strain using *E. coli–B. subtilis* shuttle vector, harboring *B.* sp. PS3 flagellin gene. The results from swarming assay with soft agar plate indicated that the R91H, G185D and G202D mutation of *B.* sp. PS3 flagellin was found to complement the *B. subtilis* flagellin mutant and the wildtype flagellin gene failed to complement. However, it was about 30% of wildtype *B. subtilis* even if most restored G185D variant. We assumed that the slight motility complementation was probably caused by short flagellar filament. In fact, short filament was confirmed by dark-field microscopic observation of vortex mixing detached flagellar structure from motility restored cell. Additionally, to ascertain functional significance of the terminally conserved hydrophobic residues, we constructed substitutions of glutamate for these hydrophobic residues.

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S3.28 Potassium and chloride channel activities from potato *Solanum tuberosum* tuber mitochondria

Izabela Koszela-Piotrowska[a], Karolina Matkovic[b], Wieslawa Jarmuszkiewicz[b], Adam Szewczyk[a]
[a]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland*
[b]*Laboratory of Bioenergetics, Adam Mickiewicz University, Poznan, Poland*
E-mail: i.piotrowska@nencki.gov.pl

Mitochondrial potassium channels, such as ATP-regulated or large conductance Ca^{2+}-activated exist both in mammals and plants. Basic effects of these channel activity include changes in mitochondrial matrix volume, mitochondrial respiration and membrane potential, and generation of reactive oxygen species. The aim of this study was to describe chloride and potassium channels from potato tuber mitochondria. Single channel activities were measured after reconstitution of the inner mitochondrial membranes into planar lipid bilayers. Three potassium channels and two chloride channels were observed. After incorporation, in a gradient of 50/450 mM KCl (*cis*/*trans*), we found that 1 mM Mg/ATP and 200–800 nM iberiotoxin (IbTx) inhibited two potassium channel activities, the ATP-regulated and the Ca^{2+}-activated with large conductance, respectively. Furthermore, it was shown that the chloride channels are inhibited by 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS). We observed also that 1 mM Mg/ATP and 400 nM 5,6-dichloro-1-ethyl-1,3dihydro-2H-benzimidazol-2-one (DCEBIO) activated one of these channels. Moreover, we found that the substances known to modulate potassium channel activities (the ATP-regulated and the Ca^{2+}-activated) influenced the bioenergetics of isolated potato tuber mitochondria, i.e., the rate of resting respiration and membrane potential.

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S3.29 Ion channels from the inner mitochondrial membrane from rat heart — single channel properties

Katarzyna Choma[a,b], Adam Szewczyk[b], Krzysztof Dołowy[a]
[a]*Department of Biophysics, Warsaw University of Life Science SGGW, 159 Nowoursynowska Street, 02-776 Warsaw, Poland*
[b]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, 3 Pasteur Street, 02-093 Warsaw, Poland*
E-mail: k.choma@nencki.gov.pl

Mitochondrial ion channels are objects of electrophysiological and pharmacological studies for over 10 years. It is known that they are involved in cytoprotection and apoptosis. In our study we investigated ion channels from the inner mitochondrial membrane of heart mitochondria. We recorded single channel activity using patch-clamp technique. An anion channel in the inner mitochondrial membrane from rat heart was observed. In symmetrical 150/150 mM KCl solution we recorded a chloride channel with conductance 120 pS. The effect of different channel inhibitors and activators (DIDS, SITS, DCEBIO) on the anion channel activity was studied. We plan to characterize its electrophysiological and pharmacological properties.

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S3.30 Cytoprotective effects of mitochondrial potassium channel opener BMS-191095

Dominika Malinska[a], Wolfram S. Kunz[b], Adam Szewczyk[a]
[a]*Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*
[b]*Department of Epileptology and Life & Brain Center, University Bonn Medical Center, Bonn, Germany*
E-mail: d.malinska@nencki.gov.pl

Mitochondrial potassium channel openers (KCO's) were shown to be cytoprotective in models of ischemia–reperfusion induced injury in brain, heart and skeletal muscle tissue. The aim of this study was to identify the cellular events responsible for observed protection. We have investigated the cytoprotective potential of BMS-191095, an opener of the mitochondrial ATP-regulated potassium channel (mitoK_{ATP}), in C2C12 myoblasts. BMS-191095 did not protect the cells against *tert*-butyl hydroperoxide or H_2O_2-induced injury, but prevented calcium ionophore A23187-induced cell death. A23187 caused a transient increase in cytosolic calcium levels, which was not affected by the presence BMS-191095. On the contrary, the opener increased the cell survival and prevented the loss of cell membrane integrity and the appearance of sub-G1 fraction observed after A23187-treatment. At comparable concentrations the opener increased respiration rate and decreased mitochondrial membrane potential of C2C12 myoblasts, which confirms that mitochondria are the site of action of this drug. Since 'mild uncoupling' of mitochondrial oxidative phosphorylation is considered as a potential mechanism of cytoprotection, these results may at least partially explain the beneficial effects of KCO's on cell survival in the conditions of disrupted calcium homeostasis.

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S3.31 A *Bacillus* flagellar motor switches from proton to sodium gradients for powering motility at alkaline pH

Naoya Terahara[a], Terry A. Krulwich[b], Masahiro Ito[a]

[a]*Graduate School of Life Sciences, Toyo University, Japan*
[b]*Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, USA*
E-mail: ito@itakura.toyo.ac.jp

Bacterial flagella contain membrane-embedded stators, Mot complexes, which harness the energy of either transmembrane H^+ or Na^+ gradients to power motility. Most bacterial stator-force generators have been shown to be coupled to the flux of H^+ whereas those of extremely alkaliphilic *Bacillus* species use Na^+ and cannot use H^+ in support of flagellar motility. There are bacteria that have two stator-force generator types (i.e. Mot complexes), one of which is coupled to H^+ while the other uses Na^+ but there have been no reports of a stator-force generator that uses both H^+ and Na^+. The genome of alkaliphilic *Bacillus clausii* KSM-K16, which is motile in a pH range from 7 to 11, encodes only one Mot (BCl-MotAB). Assays of swimming by the alkaliphile suggested that BCl-MotAB switches from proton- to sodium-coupling at high pH. This was confirmed using ion selective inhibitors of motility in swimming assays of a stator-less *Bacillus subtilis mutant expressing BCl-motAB*. By introducing distinct pairs of mutations into BCl-MotB, we constructed mutant stator forms of BCl-MotAB that no longer switch cation coupling but use either H^+ or Na^+. This work extends the range of energy-coupling options found for bacterial motility and identifies amino acid determinants of coupling-specificity.

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S3.32 Solute transporters from the *Arabidopsis* photosynthetic membrane

Cornelia Spetea[a], Lorena Ruiz Pavón[a], Sophie Thuswaldner[a], Patrik Karlsson[a], Fredrik Lundh[a], Bengt L. Persson[b]
[a]*Division of Molecular Genetics, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden*
[b]*School of Pure and Applied Natural Sciences, Kalmar University, Kalmar, Sweden*
E-mail: corsp@ifm.liu.se

The aim of this study was to identify and functionally characterize solute transporters from the chloroplast thylakoid membrane of *Arabidopsis thaliana*. As compared to chloroplast envelope transporters, much less information is available for transport processes across the thylakoid membrane, which is mostly studied as the site of light-driven photosynthetic reactions coupled to ATP synthesis. Although there are many reported examples of transport activities, only a few thylakoid transporters have been identified at the gene level. Using bioinformatics analyses, we have predicted the existence of approximately fifteen thylakoid transporters, including one ATP/ADP carrier, two phosphate transporters and one potassium channel. For experimental validation, we have carried out immuno-localization studies using peptide-specific antibodies, functional analyses in a heterologous system and phenotypic analyses of knockout mutants. The identified thylakoid ATP/ADP carrier and phosphate transporters are proposed to participate in the nucleotide metabolism in the thylakoid lumen as well as to balance the *trans*-thylakoid proton electrochemical gradient storage, whereas the potassium channel may be involved in maintaining the ionic strength of the membrane. Our data are highly relevant to understand the transport network of the thylakoid membrane and its role in photosynthesis and adaptation to environmental stress.

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S3.33 Mitochondrial large conductance potassium channel in endothelial cell

Piotr Bednarczyk[a,b], Agnieszka Łojek[a,b], Antoni Wrzosek[b], Krzysztof Dołowy[a], Adam Szewczyk[b]
[a]*Warsaw University of Life Sciences — SGGW, Department of Biophysics, Warsaw, Poland*
[b]*Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Warsaw, Poland*
E-mail: p.bednarczyk@nencki.gov.pl

It is well established that endothelial dysfunction contributes to ischemia–reperfusion injury of the cardiovascular system. This phenomenon can be limited by the ischemic preconditioning. Recently, it was shown that mitochondrial ATP regulated potassium channel activation induced ischemic preconditioning of the endothelium in humans *in vivo*. In our study a single channel activity was measured after patch-clamp of the mitoplasts isolated from endothelial cell line (EA.hy926). Mitoplast samples were prepared by addition to a hypotonic solution causing the cristae of the inner membrane to unfold and breaking of the outer membrane. Isotonicity was restored by addition of hypertonic solution. A potassium selective current was recorded with a mean conductance of 270±10 pS in symmetrical 150 mM KCl solution. Patch-clamp single channel studies showed properties of the large conductance Ca^{2+}-regulated potassium channel (BK_{Ca} channel): it was activated by calcium and NS1619 an activator of BK_{Ca} channel at micromolar concentration range. These effects were blocked irreversibly by iberiotoxin (IbTx), an inhibitor of BK_{Ca} channel. Additionally, we showed that the inhibitor of mitoK_{ATP} channel (ATP/Mg^{2+} complex) have no effects on the observed activity of the ion channel. We conclude that large conductance Ca^{2+}-regulated potassium channels are present in mitochondria isolated from endothelial cell line.

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S3.34 Mitochondrial permeability transition pore (mPTP) in different yeast species is dissimilarly regulated

Renata A. Zvyagilskaya, Marina V. Zyl'kova, Evgeniya I. Sukhanova, Mariya V. Kovaleva, Tatyana A. Trendeleva
Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia
E-mail: renata_z@inbi.ras.ru

The cyclosporin A-sensitive mPTP is considered as a major player in apoptosis in animal cells. Information about a mPTP-like pore in yeasts is scarce and contradictory. In mitochondria from the *Saccharomyces cerevisiae* yeast, the existence of unspecific channel (YMUC) inhibited upon ATP depletion was reported (see, Gutierres-Aguilar et al., 2007). The goal of this study was to investigate mPTP-like pore induction in mitochondria from the *Endomyces magnusii* and *Yarrowia lipolytica* yeasts, possessing, in contrast to *S. cerevisiae*, the fully competent respiratory chain. We failed to induce a pore mediated by Ca^{2+}-phosphate, Ca^{2+} and fatty acids, prooxidants, anaerobiosis, depletion of adenine nucleotide pools and deenergization of mitochondria. The only pore found was a regulated K^+-channel (ymitoK^+_{ATP}) of "animal type" that, in contrast to YMUC, was closed in response to ATP. Thus, mPTP in different yeast species is variously regulated.

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