

suggesting that basal proton conductance is not catalysed by all membrane proteins. These data identify a second protein that catalyses basal proton conductance in mitochondria, and support the hypothesis that this conductance is catalysed by all members of the mitochondrial anion carrier family but not by other mitochondrial inner membrane proteins.

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S3.22 Effect of large conductance calcium-activated potassium (BK_{Ca}) channel openers on endothelial mitochondria

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The aim of this study was to determine effects of NS1619 and CGS7184 BK_{Ca} channel openers on oxygen consumption, mitochondrial membrane potential and calcium homeostasis of endothelial cells EA.hy926. CGS7184 caused acceleration of cell respiration, whereas NS1619 lowered it. Both compounds induced a drop in mitochondrial membrane potential and caused increase in Ca²⁺ level. Subsequent addition of NS1619 and CGS7184 caused additional increase in [Ca²⁺]_i, which suggests different molecular targets for these substances. Discrepancies were observed when FURA-2 fluorescence was quenched with Mn²⁺. In vascular preparations of isolated mice heart NS1619 and CGS7184 induced coronary vasodilation, but involvement of NO was more pronounced for the response induced by CGS7184 as compared with NS1619. Our results suggest that apart from potassium channels opening properties CGS7184 and NS1619 possess distinct pleiotropic actions on EA.hy926 cells causing increase or decrease in the respiration rate, changes in mitochondrial membrane potential and alterations in intracellular calcium homeostasis that may explain different NO-releasing potency of NS1619 and CGS7184 in vascular preparations.

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S3.23 Cyclophilin D sensitizes the mitochondrial permeability transition to phosphate

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Mitochondria isolated from mice with inactivation of *Ppif*, the unique gene encoding for mitochondrial cyclophilin D (CyPD), accumulate larger loads of Ca²⁺ than mitochondria from wild-type (WT) animals before undergoing the permeability transition (PT), i. e. they have a higher Calcium Retention Capacity (CRC). We show

here that this remarkable property of CyPD-null mitochondria is not due to a decreased sensitivity of the mitochondrial permeability transition pore (PTP) to Ca²⁺, but rather to an effect of the inorganic phosphate (Pi) which is taken up in parallel. When Pi was replaced by anions with similar properties that also allow Ca²⁺ accumulation (such as arsenate and vanadate), the CRC was the same in WT and CyPD-null mitochondria. Thus, CyPD sensitizes the PTP to Pi rather than Ca²⁺, a finding that has major implications for our understanding of the effects of CyPD on PTP modulation *in vivo*.

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S3.24 The atypical plasmalemmal dicarboxylate transporter of *Saccharomyces cerevisiae*

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The aim of this study was to characterize the putative dicarboxylate transporter in the plasma membrane of *S. cerevisiae*: its substrate specificity, kinetic properties, and mechanism. Transport of succinate and citrate into *S. cerevisiae* cells has been measured by monitoring oxidation rates of these substrates. Linearity of the Dixon plot obtained with impermeable effective competitive inhibitor 2-undecylmalonate suggests that it blocked plasmalemmal transport upon oxidation of both substrates. In the monosodium incubation medium, the K_m value for succinate oxidation (transport) decreased with increasing pH value, thus suggesting that succinate is predominantly transported in the dianionic form. Influx of succinate and citrate at pH 5.5 was insensitive to the protonophore FCCP, competitively inhibited by 2-undecylmalonate (with close K_i values for both substrates). This suggests that both citrate and succinate entered the cell via a common plasma membrane transporter, which is atypical for fungi. Mechanisms of functioning of transporter, as dicarboxylate-proton symport or ATP-dependent transport were excluded. Highly improbable was cation-supported substrate symport. Low activity and the wide substrate specificity of transporter (succinate, malate, citrate, malonate) permit to exclude a role of this carrier as a substrate sensor. Kinetic properties of the transporter are not contradictory to the facilitated diffusion mechanism.

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S3.25 Complementation of *Bacillus subtilis* motility with flagellin gene from thermophilic *Bacillus* sp. PS3

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Flagellation is widespread in bacteria or archaea. The principal component of bacterial flagellum is the long helical filament which comprises ~20,000 flagellin subunits. Flagellins from *Bacillus* sp. PS3 consist of variable central region and highly conserved both terminal regions, which have hepta-hydrophobic amino acid repeats and it was suggested to be important to filament assembly.

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

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Mitochondrial potassium channels, such as ATP-regulated or large conductance Ca²⁺-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 Ca²⁺-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-1,3-dihydro-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 Ca²⁺-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

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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

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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 H₂O₂-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

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