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

Antoni Wrzosek[b], Agnieszka Łojek[a,b], Krzysztof Dołowy[a], Paweł Gwóźdź[c], Stefan Chłopicki[c], Adam Szewczyk[b]
[a]*Department of Biophysics, University Life of Sciences—SGGW, 159 Nowoursynowska Street, 02-776 Warsaw, Poland*
[b]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland*
[c]*Department of Experimental Pharmacology, Chair of Pharmacology, Medical College of Jagiellonian University, 16 Grzegórzecka, 31-531 Kraków, Poland*
*E-mail:* a.wrzosek@nencki.gov.pl

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 $Ca^{2+}$ level. Subsequent addition of NS1619 and CGS7184 caused additional increase in $[Ca^{2+}]_i$, which suggests different molecular targets for these substances. Discrepancies were observed when FURA-2 fluorescence was quenched with $Mn^{2+}$. 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

Emy Basso[a], Valeria Petronilli[a], Mike Forte[b], Paolo Bernardi[a]
[a]*Department of Biomedical Sciences, University of Padova, 35131 Padova, Italy*
[b]*Vollum Institute, Oregon Health and Sciences University, Portland, Oregon, USA*
*E-mail:* basso@bio.unipd.it

Mitochondria isolated from mice with inactivation of *Ppif*, the unique gene encoding for mitochondrial cyclophilin D (CyPD), accumulate larger loads of $Ca^{2+}$ 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 $Ca^{2+}$, 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 $Ca^{2+}$ 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 $Ca^{2+}$, 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*

Dinara A. Aliverdieva[a,b], Dmitry V. Mamaev[a], Dmitry I. Bondarenko[a], Kristophor F. Sholtz[a], Renata A. Zvyagilskaya[a]
[a]*A. N. Bach Institute of Biochemistry, Russian Academy of Science, Moscow, Russia*
[b]*Caspian Institute of Biological Resources, Russian Academy of Science, Russia*
*E-mail:* dinara_inbi@mail.ru

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

Junpei Hayakawa, Morio Ishizuka
*Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, Tokyo, Japan*
*E-mail:* jhykw1979@yahoo.co.jp

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.