S3.18 Redox state of quinone affects *Acanthamoeba castellanii* mitochondrial uncoupling protein activity through sensitivity to purine nucleotides

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We studied free fatty acid-induced uncoupling activity in *A. castellanii* mitochondria in nonphosphorylating state. Either succinate or external NADH was used as a respiratory substrate to determine the proton conductance curves and the respiratory rate versus the quinone reduction level relationships. Our determinations of the membranous quinone reduction level in nonphosphorylating mitochondria show that activation of UCP activity leads to a purine nucleotide-sensitive decrease in quinone redox state. The gradual decrease in the rate of quinone-reducing pathways (titration of dehydrogenases activity) progressively leads to a full inhibitory effect of GDP on linoleic-acid induced proton conductance. This inhibition cannot be attributed to changes in the membrane potential. Indeed, the lack of GDP inhibitory effect observed when decrease in respiratory rate is accompanied by an increase in the quinone reduction level (titration of quinol-oxidizing pathway) proves that the inhibition by nucleotides can be revealed only for a low quinone redox state. It must be underlined that in *A. castellanii* nonphosphorylating mitochondria, the transition of the inhibitory effect of GDP on linoleic acid-induced UCP-mediated uncoupling is observed for the same range of quinone reduction level (between 50% and 40%) as that observed previously for phosphorylating conditions. This observation drawn from the two different metabolic states of mitochondria indicates that quinone could directly affect UCP activity.

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S3.19 Mitochondrial free $[Ca^{2+}]$ and the permeability transition pore

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Mitochondrial Ca^{2+} activates many processes, from mitochondrial metabolism to opening of the permeability transition pore (PTP) and apoptosis. However, while the micromolar Ca^{2+}-dependence of mitochondrial dehydrogenases is well known, the mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_M$) required to trigger opening of the PTP has not been established. There is also considerable controversy regarding the free $[Ca^{2+}]_M$ levels. Studies using high-Ca^{2+}-affinity fluorescent dyes (rhod-2), have reported that phosphate precipitation precludes mitochondrial $[Ca^{2+}]$ from increasing above 2–3 μM. Instead, using low-Ca^{2+}-affinity aequorin probes, we have measured $[Ca^{2+}]_M$ values more than two orders of magnitude higher. We show here that a prolonged increase in $[Ca^{2+}]_M$ to levels of 200–800 μM was actually observed at any phosphate concentration (0–10 mM) during continuous perfusion of 2–10 μM Ca^{2+} buffers. Direct *in situ* calibration of mitochondrial aequorin showed the same Ca^{2+}-dependence as that previously reported in endoplasmic reticulum. A full, rapid and reversible drop in $[Ca^{2+}]_M$ was observed after the addition of a protonophore. However, this high and maintained $[Ca^{2+}]_M$ did not induce opening of PTP unless additional activators such as phenyl arsine oxide were added, this leading to a rapid and concentration-dependent drop in $[Ca^{2+}]_M$. In conclusion, mitochondrial $[Ca^{2+}]$ levels above 100 μM can be reached and maintained for prolonged periods (>15 min) in phosphate-containing medium, and massive opening of PTP requires the presence of additional pore activators.

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S3.20 Regulation of the energy-dissipating systems in *Acanthamoeba castellanii* mitochondria by purine nucleotides

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Mitochondria of *Acanthamoeba castellanii* (*Protozoa*) possess energy-dissipating systems: (i) a cyanide-resistant alternative oxidase (AOX), (ii) a free fatty acid-activated uncoupling protein (UCP), and recently discovered (iii) an ATP-sensitive potassium channel (mitoK_{ATP}). The activity of these systems, thereby the efficiency of oxidative phosphorylation, is regulated by guanosine and adenosine 5′-phosphates in a different way. The activity of the AOX is stimulated significantly by guanine nucleotides (GMP, GDP, and GTP) and slightly by adenine nucleotides (AMP and ADP). By contrast the activity of the UCP is inhibited by the tri- and diphosphate nucleosides, while the inhibition by monophosphate nucleosides is negligible. On the other hand, in the case of the mitoK_{ATP}, its sensitivity to purine nucleotides is not yet well described besides the inhibition by ATP. It is noteworthy that in *A. castellanii* mitochondria, ATP inhibits all these three systems including the alternative oxidase (reported here for the first time). Therefore it indicates that ATP is a key modulator in maintaining the energetic and metabolic balance during the life of this unicellular organism.

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S3.21 Contribution of mitochondrial carrier proteins to basal proton conductance

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The coupling of substrate oxidation to ATP synthesis in mitochondria is incomplete because of proton leak pathways across the mitochondrial inner membrane. These pathways can be classified as basal (not regulated) or inducible (regulated by activators and inhibitors). Here we examine the contributors to basal proton conductance. Half to two-thirds of basal proton conductance is catalysed by the abundant inner membrane carrier adenine nucleotide translocase (ANT). To determine whether ANT is the unique protein catalyst or one of many proteins that catalyse basal proton conductance, we examined proton conductance in brown adipose tissue mitochondria, which express another mitochondrial carrier (UCP1) at concentrations higher than ANT. Basal proton conductance was measured under conditions in which UCP1 was catalytically inactive (high serum albumin and presence of GDP) and was significantly lower in mitochondria from UCP1 knockout mice than wild-type showing UCP1 can also catalyse basal proton conductance. We also tested whether non-carrier mitochondrial proteins catalyse this reaction. Ablation of another abundant inner membrane protein, nicotinamide nucleotide transhydrogenase, showed no effect on basal proton conductance,

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 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

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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 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*

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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.