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,