Reference
[1] Koder RL *et al.* (2009) *Nature* **458:** 305–309.

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5P.18 Characterization of the permeability transition pore in mitoplasts exposed to photooxidative stress

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Hematoporphyrin (HP)-mediated photooxidative stress can either prevent or activate the mitochondrial permeability transition (PT) depending on the site of porphyrin/target localization and on the light dose. Under irradiation with moderate light doses, HP situated in matrix-exposed sites of the PT pore (PTP) promotes photosensitization of key His residues leading to PT inhibition. Under irradiation with high light doses, PT is re-activated through photomodification of external Cys residues by vicinal HP. Here we checked whether the peculiar photosensitizing properties of HP on the PTP were maintained in mitoplasts (inner membrane preparations) obtained by treatment with proper digitonin concentrations. Mitoplast purity was verified by enzymatic analysis of the outer membrane marker monoamino oxidase and by electron microscopy. In analogy with the results obtained in intact mitochondria, irradiation of HP-treated mitoplasts at low light doses caused PT inhibition that was counteracted by diethyl pyrocarbonate, indicating that it resulted from photomodification of PTP-regulating His residues. At variance from mitochondria, however, in mitoplasts the PT could not be reactivated after exposure to prolonged irradiation periods, yet opening of a CsA-sensitive PTP could be still observed upon addition of the membrane-impermeant, thiol-oxidant copper-*o*-phenanthroline [$Cu(OP)_2$], indicating that mitoplasts retain the external PTP-regulating sulfhydryls. Ablation of PT re-activation in mitoplasts was specific for dicarboxylic porphyrins endowed with protoporphyrin IX (PP) configuration, such as deuteroporphyrin (DP) and PP itself, which exhibit nanomolar affinity for the outer membrane-associated translocator protein of 18 kDa (TSPO, formerly called peripheral benzodiazepine receptor); whereas PP-unrelated porphyrins did not affect mitochondria or mitoplasts under irradiation. We suggest that in intact mitochondria thiol-sensitizing HP interferes with the PTP through interaction with specific regions of the TSPO.

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5P.19 Chronological lifespan extension mediated by calorie restriction in *Saccharomyces cerevisiae* requires mitochondrial electron transport chain integrity

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Aging can be defined as a progressive decline in metabolic function and efficiency of biological systems over time [1]. Our group elected *Saccharomyces cerevisiae* as a model organism and calorie restriction (CR) as a nutritional intervention to uncover which are the most relevant mitochondrial aging hallmarks in eukaryotic cells [2–4]. Since glucose availability in YPD media is finite, oxidative metabolism becomes essential to maintain *S. cerevisiae* viability when in the stationary phase [5]. We determined glucose exhaustion by the use of a refraction-index detector coupled to an HPLC in standard (2.0%) and glucose-restricted (0.5%) YPD and observed that glucose is totally consumed after 24 h and 18 h, respectively. In order to investigate the role of aerobic metabolism on *S. cerevisiae* chronological viability, which involves the study of electron transport chain constituents and the mitochondrial genome, we measured chronological lifespan for 28 days in *lpd1Δ S. cerevisiae* (mutants that do not display pyruvate and α-ketoglutarate dehydrogenase activities), *abf2Δ* (mutants that present marked mitochondrial genome instability), *cyt1Δ* (mutants which do not produce cytochrome c_1) and ρ-/0 (mutants in which mtDNA is partially or totally absent) through colony-forming ability in YPD plates [3, 4]. We observed that all mutants studied presented decreased chronological lifespans when compared to WT. We also found that, unlike *abf2Δ*, *cyt1Δ* and ρ-/0 mutants, *lpd1Δ S. cerevisiae* responded to CR by increasing chronological lifespan and, surprisingly, exhibited a residual respiratory growth capacity. Altogether, our data present relevant evidence that citric acid cycle disruption in α-ketoglutarate dehydrogenase does not abolish lifespan extension in response to CR and that respiratory growth capacity – provided by mitochondrial electron transport chain functional integrity – is closely related to increased chronological viability promoted by CR in *S. cerevisiae*.

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References
[1] Jazwinski SM (2002) *Annu. Rev. Microbiol.* **56:** 769–792.
[2] Barros MH *et al.* (2004) *J. Biol. Chem.* **279:** 49883–49888.
[3] Tahara EB *et al.* (2007) *FASEB J.* **21:** 274–283.
[4] Oliveira GA *et al.* (2008) *J. Bioenerg. Biomembr.* **40:** 381–388.
[5] Fabrizio P and Longo VD (2007) *Methods Mol. Biol.* **371:** 89–95.

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5P.20 Effect of fatty acids and mitochondria-targeted lipophilic cations on yeast mitochondria

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The effect of fatty acids and lipophilic cations (SkQ1, SkQ3, MitoQ, and C_{12}TPP, originally designed as mitochondria-targeted antioxidants), on tightly-coupled yeasts mitochondria was investigated. Micromolar concentrations of saturated and unsaturated fatty acids were found to decrease the membrane potential, which was recovered almost totally by ATP and BSA. At low, micromolar concentrations, mitochondria-targeted lipophilic cations are mild uncouplers, at higher concentrations they inhibit respiration in state 3, and at much higher concentrations they induce swelling of mitochondria, possibly due to their prooxidant and detergent action. At very low, not uncoupling concentrations, mitochondria-targeted lipophilic cations profoundly promote the uncoupling effect of fatty acids. The mechanism underlying this process is proposed. It is conceivable that the observed uncoupling effect of lipophilic cations

can be, at least partially, due to their interactions with the endogenous pool of fatty acids.

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5P.21 The effect of Ca^{2+} on reactive oxygen species generation in brain mitochondria in the absence of permeability transition

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Glutamate excitotoxicity is a key element in the pathomechanism of acute (ischemia–reperfusion) and chronic (Alzheimer's disease, Parkinson's disease, Huntington's disease) neurological disorders. Stimulation of glutamate receptors results in the elevation of intracellular [Ca^{2+}] which can activate reactive oxygen species (ROS) generation. The aim of our experiments was to study the effects of high micromolar Ca^{2+} concentrations on the H_2O_2 generation in isolated guinea pig brain mitochondria, supported by NADH-generating substrates; glutamate plus malate. H_2O_2 formation was detected extramitochondrially by Amplex red assay. In parallel with ROS formation NAD(P)H autofluorescence was detected. Mitochondrial membrane potential ($\Delta\psi_m$) was measured by safranine O and TMRM fluorescence respectively. Swelling of mitochondria was detected by light scattering. Permeability transition pore (PTP) opening was measured by calcium induced calcium release and by quenching of calcein fluorescence. PTP was prevented by ADP, a very efficient inhibitor of mitochondrial permeability transition. In the presence of ADP 50 μM Ca^{2+} (500 nmol/mg protein) did not induce PTP opening but enhanced mitochondrial H_2O_2 release by 81 ± 18%. Mitochondria were able to take up calcium; after a transient depolarization $\Delta\psi_m$ was restored, even hyperpolarization was detected and parallel with these NAD(P)H fluorescence was increased. With 300 μM Ca^{2+} membrane potential collapsed without recovery and H_2O_2 release was unchanged. At 300 μM [Ca^{2+}] in the presence of ADP, mitochondria were unable to complete Ca^{2+}-uptake but no signs of PTP were detected in the time-frame of the experiments. In highly polarized mitochondria, in the presence of ATP or oligomycin ROS production was elevated, Ca^{2+} failed to stimulate mitochondrial ROS generation and hyperpolarization did not follow the Ca^{2+}-induced depolarization. It is suggested that in the presence of nucleotides the effect of Ca^{2+} on mitochondrial ROS release is related to changes in $\Delta\psi_m$. The increased ROS release evoked by Ca^{2+} in the presence of ADP in isolated mitochondria is unrelated to PTP and would not explain the extensive cellular ROS production observed during glutamate excitotoxicity.

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5P.22 Cardiolipin: Altered content and fatty acid composition in mitochondria from mtDNA mutator mice

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We have recently demonstrated that the assembly and turnover of the mitochondrial respiratory chain complexes I, III and IV (but not complexes II and V) are altered in mitochondria from mtDNA mutator mice [1]. Since it is known that cardiolipin is essential for assembly and stability of respiratory chain complexes, we have elected to study this phospholipid in mitochondria from mtDNA mutator mice. The content of mitochondrial phospholipids was analysed by two-dimensional high performance thin layer chromatography (2D-HPTLC). The content of cardiolipin was significantly lower in liver and skeletal muscle mitochondria from mtDNA mutator mice than in wild-type mitochondria. To analyse the fatty acid composition of cardiolipin, gas chromatography/flame ionization detection or electron ionization-mass spectrometry (GC/FID or EI MS) was applied. The content of the polyunsaturated *n-6* fatty acids was remarkable lowered in the cardiolipin fraction from skeletal muscle and liver mitochondria of mtDNA mutator mice, as compared with wild-type mice. Mitochondrial phospholipids were also studied in mice chronically treated with mitochondria-targeted antioxidant (plastoquinone derivative, SkQ1), added to drinking water. The content of cardiolipin and its fatty acid composition were normalised in mtDNA mutator mitochondria after treatment with SkQ1. We conclude that cardiolipin content is decreased and its fatty acid composition markedly altered in mitochondria from mtDNA mutator mice. The cause and significance of these alterations are of interest, considering the special role of cardiolipin in mitochondrial bioenergetics.

Reference

[1] Edgar D *et al.* (2009) *Cell. Metab.* **10:** 131–138.

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5P.23 Antioxidant defence systems and generation of reactive oxygen species in osteosarcoma cells with defective mitochondria: Effect of selenium

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Mitochondrial diseases originate from mutations in mitochondrial or nuclear genes encoding for mitochondrial proteome. Neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) syndrome is associated with the T8993G transversion in *ATP6* gene which results in substitution at the very conservative site in the subunit 6 of mitochondrial ATP synthase. Defects in the mitochondrial respiratory chain and the ATPase are considered to be accompanied by changes in the generation of reactive oxygen species (ROS). This study was aimed to elucidate effects of selenium on ROS and antioxidant system of NARP cybrid cells with 98% of T8993G mutation load. We found that selenium decreased ROS generation and increased the level and activity of antioxidant enzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). Therefore, we propose selenium to be a promising therapeutic agent not only in the case of NARP syndrome but also other diseases associated with mitochondrial dysfunctions and oxidative stress.

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5P.24 Response of *Acanthamoeba castellanii* mitochondria to hydrogen peroxide stress

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