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

Justina Šileikytė[1], Valeria Petronilli[1], Paolo Bernardi[1], Fernanda Ricchelli[2]
[1]*C.N.R. Institute of Neurosciences at the Department of Biomedical Sciences, University of Padova, Italy*
[2]*C.N.R. Institute of Biomedical Technologies at the Department of Biology, University of Padova, Italy*
E-mail: rchielli@mail.bio.unipd.it

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

Erich B. Tahara[1], Kizzy Cezário[2], Mario H. Barros[2], Nadja C. Souza-Pinto[1], Andreas K. Gombert[3], Alicia J. Kowaltowski[1]
[1]*Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil*
[2]*Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil*
[3]*Departamento de Engenharia Química, Escola Politécnica, Universidade de São Paulo, Brazil*
E-mail: alicia@iq.usp.br

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

Tatyana A. Trendeleva, Evgeniya I. Sukhanova, Renata A. Zvyagilskaya
A.N. Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russian Federation
E-mail: tatiana_tren@mail.ru

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