mechanisms by which mitochondrial enzymes generate toxic free radicals. Due to its low potential cofactors, mitochondrial complex I is a prime candidate for significant radical production. Indeed, studies on both isolated complex I [1] and intact mitochondria [2] have shown that complex I can generate significant levels of the reactive oxygen species (ROS) superoxide and hydrogen peroxide. During NADH oxidation, studies of the isolated enzyme have described a single site of ROS production (the flavin mononucleotide). Conversely work on intact mitochondria has suggested that a second site contributes during NADH oxidation, and the locus of ROS production during reverse catalysis is unclear. Reverse catalysis is not possible with the isolated enzyme. To resolve the mechanisms of ROS production in both directions of catalysis we have prepared tightly coupled submitochondrial particles (SMPs) from bovine heart mitochondria. Because they are inside out with respect to mitochondria we have direct access to the catalytic sites of the respiratory complexes, and the ability to detect the ROS produced directly (without interference from any antioxidant protection systems). Here, we describe how ROS production by complex I responds to the NAD^+/NADH ratio, to the presence of inhibitors, and to the proton motive force. The influence of semiquinone intermediates is explored during catalytic turnover. Consequently, we provide a unified molecular mechanism for ROS production by complex I.

References

[1] Kussmaul L, Hirst J (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103:** 7607–7612.
[2] Lambert AJ, Brand MD (2004) *J. Biol. Chem.* **279:** 39414–39420.

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5P.16 Growing fast and dying young: A mitochondrial coupling problem?

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Organism body size is known to be positively correlated with longevity at least at inter-specific level. However, the interplay between growth and senescence is still poorly documented at the intra-specific level, especially in ectotherms. Here, we present an intra-species comparison of two neighbouring populations of frogs (*Rana temporaria*) that present large differences in both body mass (2–3 fold, at same age) and lifespan (being shorter in large morph than in small one). In the light of the mitochondrial free radical theory of aging, we hypothesised that an alteration in the mitochondrial functioning would play a part in differential growth rates and survival. Thus, we assessed key parameters of frog's liver mitochondria from both populations enabling a comparison between fast and low growth rate phenotype (hereafter called fast GR and low GR).Our data shows that the efficiency of oxidative phosphorylation process (ATP/O ratio), in liver mitochondria, was three-fold higher in fast GR frogs than in low GR ones ($P<0.05$). However, no age effect (with 3, 4 and 5 years-old individuals) was demonstrated on ATP/O ratio, neither in low nor in fast GR. Interestingly, phosphorylating (State 3) and non phosphorylating (State 4) respiration rates were identical in both populations ($P=0.87$ and $P=0.30$, respectively) while the maximal rate of ATP synthesis was 2.4-fold higher in liver mitochondria of fast GR than in low GR phenotype (2.15 ± 0.16 *vs.* 0.91 ± 0.24 nmol ATP/min mg protein, respectively; $P<0.05$).As Cytochrome Oxidase activity remained unchanged in liver mitochondria from both frog populations it could not explain these original results. Nevertheless, the higher rate of ATP hydrolysis by the ATP synthase complex observed in fast GR phenotype (62.82 ± 10.75 *vs.* 33.34 ± 7.24 nmol ATP/min mg protein, fast GR and low GR phenotype, respectively; $P<0.05$) could partly explain our results. For the first time, we describe an important age-independent association between mitochondrial plasticity (affecting the ATP production) and growth rate. It is now important to describe how such plasticity, which affects the efficiency of oxidative phosphorylation process, impact on ROS production and antioxidant defenses.

Keywords: Amphibian, lifespan, growth rate, mitochondria, ATP/O ratio.

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5P.17 Design and engineering of superoxide oxidoreductase activity in new artificial proteins

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Superoxide(SO) is a ubiquitous product or by product among cellular metabolic reactions, though many sources remain to be identified. Its production may be purposeful as a cell-signaling molecule, through conversion to hydrogen peroxide, or as a bactericidal agent used by neutrophils. However, SO can also have deleterious effects, especially after converting to more potent radicals *via* peroxide or nitrous oxide. These ROS are implicated in oxidative stress and age-related cellular malfunctions. Resolution of ROS production at specific cofactor sites within implicated proteins, such as Complexes I and III, has been elusive. This is most likely due to the presence of multiple sites of generation. To address this challenge we use step wise engineering approaches to design artificial enzymes that can resolve the mechanisms of ROS production at individual sites. Artificial 4-helixbundle proteins have been synthesized that ligate hemes, flavins and quinones, allowing for 1 or 2 electron transfer (ET) to O_2. So far, results have been obtained for heme proteins. Flavin- and quinone-containing proteins will soon be studied for their SO-generating activities. Two heme-ligating variants were derived from an artificial oxygen transport protein [1] to examine two means of SO generation: inner-and outer-sphere ET. The parent protein bound O_2 stably and SO generation was undetectable. One variant was redesigned to render the heme water-accessible. This destabilized the oxyferrous state and yields SO by inner-sphere ET as in globins. The other variant was redesigned to lack strain essential for O_2-binding but to retain water-inaccessibility. As designed, it failed to bind O_2 and yielded SO by outer-sphere ET. Both variants produce SO at rate that matches SO-generating enzymes such as NADPH oxidases. SO was detected with the SO-specific chemiluminescent probe methyl-cypridina-luciferin analogue (MCLA). SO generation was monitored by stopped-flow while heme oxidation was monitored independently by UV–Visible spectroscopy. This work demonstrates the design and engineering of multiple mechanisms of ROS production in artificial proteins that display catalytic activity approaching that of natural enzymes.

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