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Several lines of evidence have suggested that energy metabolism regulation deeply related to longevity of organisms. For example, most age-related genes were correlated with energy metabolism. Also, dysfunction of mitochondria increases longevity of several organisms. However, relationship between aging and production/consumption of ATP in organisms has been rarely known. In this study, we have attempted to determine the change of ATP concentration during aging in nematodes by both bulk phase and molecular imaging analysis. We report the change of ATP concentration during aging in nematode.

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**5P.12 Comparison of superoxide production of rat brain mitochondria analyzed with hydroethidine and MitoSOX**

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The production of superoxide generation is implicated for several types of neurodegenerative diseases and aging. Despite the progress in characterising the ROS effects on cell function, the mechanisms of cellular superoxide formation are less well understood. Considerable difficulties and artefacts are observed with different methods for detection of ROS, and in particular superoxide. In this work we compared MitoSOX and Hydroethidine, the well known dyes for superoxide detection in living cells and tissue slices, for the suitability to detect superoxide in isolated rat brain mitochondria. Hydroethidine (HET) is used to visualize superoxide localized in cytoplasm. For more targeted detection of ROS production in the mitochondria, hydroethidine is modified by conjugating this dye to triphenylphosphonium (MitoSOX). We observed that, unlike hydroethidine, MitoSOX allows to detect superoxide generation in isolated rat brain mitochondria respiring on the complex II substrate succinate. This superoxide generation, detected by MitoSOX, was sensitive to uncoupler and rotenone. This indicates that it is due to reversed electron flow caused ROS generation by Complex I. Similarly, rotenone increased the superoxide generation detected by MitoSOX but not HET in the presence of the complex I substrates glutamate + malate, α-ketoglutarate and pyruvate + malate. Since the MitoSOX is assumed to be accumulated at the inner side of mitochondrial inner membrane, this indicates that Hydroethidine and MitoSOX probably detect mitochondrial superoxide production in different local compartments.

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**5P.13 Tissue specific effects of MnSOD knockout in mice**

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A critical role of mitochondrial dysfunction and oxidative damage has been hypothesized in both aging and neurodegenerative diseases. Mitochondria are the main source of reactive oxygen species in cells because 2–4% of the oxygen consumed by mitochondria is converted to superoxide anions by the electron transport chain and moreover mitochondria have restricted protection against oxidative stress. To determine the importance of mitochondrial oxygen species toxicity, we analyzed heart muscle tissue and fibroblasts from mutant mice, with deficiencies in the mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD) generated in three different ways. The knockout mouse models have been produced by disruption of different regions of Sod2 gene (Li Y *et al.* (1995) *Nat. Genet.* **11**: 376–381; Lebovitz RM *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**: 9782–9787). In agreement with literature in heart muscle we observed aconitase deficiency (Melov S *et al.* (1999) *Proc. Natl. Acad. Sci. USA* **96**: 846–851). In comparison with wild type animals the knockout mice showed only one third of aconitase activity. In contrast, the fibroblast cultures from these mice did not show any alteration of aconitase activity. In the digitonin treated fibroblast the resting state of respiration was elevated, while active state respiration was not affected. Our findings demonstrate strong tissue specificity effect of MnSOD knockout.

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**5P.14 Clear-up of Redox state under hypoxia**

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Hypoxic adaptations participate in numerous physiological (i.e. strenuous exercise) and pathological situations (i.e. ischemia and tumor development). The mitochondria, as the oxygen sensor, serve as a primary regulatory element within this process. By-products of mitochondrial metabolism, free radicals or reactive oxygen and nitrogen species (ROS/RNS), participate, in balance with antioxidant shield, in redox status of the cell. Redox signaling was shown to be a principal regulator of metabolic responses to low oxygen, mainly through HIF1-mediated reprogramming of gene expression. We have attempted here to clarify the reported controversies in ROS/RNS production during various stages of hypoxic adaptations. We have found that the amount of ROS/RNS production differs in the course of hypoxic adaptation and reflects involvement of mitochondrial metabolism. Moreover, we have located the production sites of ROS/RNS and characterized also selected scavenging system. Finally, we have correlated the redox status with mitochondrial metabolism and morphology.

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**5P.15 Do semiquinones formed by mitochondrial complex I contribute to reactive oxygen species production?**

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Mitochondria have been identified as a major source of the oxidative and nitrosative stresses that can compromise cellular homeostasis. Mutations in several mitochondrial enzymes are now recognised as the cause of disease states, and severe mitochondrial dysfunction and elevated radical production are implicated in neurodegenerative pathologies including Alzheimer's and Parkinson's. Therefore, it is important to characterise the sites and

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.

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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 ($2.15\pm0.16$ *vs.* $0.91\pm0.24$ 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 ($62.82\pm10.75$ *vs.* $33.34\pm7.24$ 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.