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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