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5L.7 What are the sources of hydrogen peroxide production by heart mitochondria?

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Several mitochondrial enzymes, the respiratory complexes I and II, oxoglutarate dehydrogenase, free dihydrolipoamide dehydrogenase, and monoamine oxidase are potential contributors to overall intra- and extramitochondrial production of hydrogen peroxide generated either directly or via intermediate formation of superoxide anion. At least three intramitochondrial enzymes, Mn-superoxide dismutase, glutathione peroxidase, and catalase are involved in further reduction of superoxide and H_2O_2. The intramitochondrial steady-state level of hydrogen peroxide and its external production are thus resulted from an interplay between these enzyme activities. We measured the rates of H_2O_2 and superoxide generation by heart mitochondrial preparations of different degree of resolution: (i) intact coupled mitochondria, (ii) inside-out submitochondrial particles (SMP), (iii) alamethicin-treated mitochondria (A-mito, uncoupled mitochondria, permeable for low mol. mass components), and (iv) soluble matrix proteins and purified fractions derived there from. The NADH- and succinate-supported superoxide generation by SMP are strongly suppressed at high physiologically relevant concentrations of either NADH or NAD^+. Hydrogen peroxide formation by A-mito assayed under optimal conditions for complex I-mediated reaction (low NADH in the presence of rotenone) is only partially sensitive to complex I-specific active site-directed inhibitor, NADH-OH. The residual inhibitor-insensitive activity is strongly and specifically stimulated by NH_4^+. A soluble matrix located protein fraction (mol. mass of about 50 kDa) responsible for the ammonia-dependent NAD(P)H-supported hydrogen peroxide formation was purified. It catalyzes NADH:lipoamide and NADPH:glutathione oxidoreductase reactions and shows significant homology with dihydrolipoamide dehydrogenase. The data suggest that in heart mitochondria the soluble matrix located protein(s), not the respiratory chain components, are the major contributor(s) to hydrogen peroxide formation. Whether relative contributions of the respiratory chain components and matrix located flavoproteins to the extra- and intramitochondrial hydrogen peroxide production is the same in other than heart tissues remain to be established.

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5L.8 Oxidative stress-dependent p66Shc phosphorylation in skin fibroblasts of children with mitochondrial disorders

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p66Shc, the growth factor adaptor protein, can have a substantial impact on mitochondrial metabolism through regulation of cellular response to oxidative stress. We investigated relationships between the extent of p66Shc phosphorylation at Ser36, mitochondrial dysfunctions and an antioxidant defence reactions in fibroblasts derived from five patients with various mitochondrial disorders (two with mitochondrial DNA mutations and three with methylglutaconic aciduria and genetic defects localized, most probably, in nuclear genes). We found that in all these fibroblasts the extent of p66Shc phosphorylation at Ser36 was significantly increased. This correlated with a substantially decreased level of mitochondrial superoxide dismutase (SOD2) in these cells. This suggest that SOD2 is under control of the Ser36 phosphorylation status of p66 protein. As a consequence, an intracellular oxidative stress and accumulation of damages caused by oxygen free radicals are observed in the cells.

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Posters

5P.1 Inhibition of the α-ketoglutarate dehydrogenase-mediated reactive oxygen species generation by lipoic acid

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Dihydrolipoamide dehydrogenase (LADH) is a flavo-enzyme that serves as a subunit of α-ketoglutarate dehydrogenase complex (α-KGDHC). Reactive oxygen species (ROS) generation by α-KGDHC has been assigned to LADH (E3-subunit) and explained by the diaphorase activity of E3. Dysfunctions of α-KGDHC and concurrent ROS-production have been implicated in neurodegeneration, ischemia–reperfusion and other pathological conditions. In this work we investigated the intimate details of ROS-generation by isolated LADH and α-KGDHC. We found a parallel generation of superoxide and hydrogen peroxide by the E3-subunit of α-KGDHC which could be blocked by lipoic acid (LA) acting on a site upstream of the E3-subunit. The pathologically relevant ROS-generation (at high NADH/NAD + ratio and low pH) in the reverse mode of α-KGDHC could also be inhibited by LA. Our results contradict the previously proposed mechanism for pH-dependent ROS-generation by LADH, showing no disassembling of the E3 functional homodimer at acidic pH using a physiologically relevant method for the examination. It is also suggested that LA could be beneficial in reducing the cell damage related to excessive ROS-generation under pathological conditions.

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5P.2 Measuring mitochondria-derived reactive oxygen species in cell culture: Challenges and limitations

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Mitochondrial reactive oxygen species (ROS) are not only involved in the pathophysiology of many diseases, increasing evidence also suggests an important role in cellular redox signaling. Most studies to date have used isolated mitochondria for kinetic measurements of the mitochondrial ROS production or cellular ROS generation has been detected by an end point determination. Considering the highly dynamic regulation of mitochondrial ROS generation, kinetic measurements in cultured cells would be preferable, because this would represent a situation closer to physiological conditions and would allow the investigation of crosstalk between mitochondria and cytoplasmic components. Thus our aim was to identify reliable ROS detection assays for kinetic measurements in cell culture. We tested the applicability of several commonly used assays with different cell types and r^0 cells by analyzing the effects of known effectors of mitochondrial ROS generation (inhibitors of respiratory chain complexes, uncouplers). In a comparative study similar measurements were done with isolated mitochondria and permeabilized cells. The advantages and disadvantages of each of the tested methods will be discussed.

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5P.3 Proteomic evolution of *Saccharomyces cerevisiae* during chronological aging

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Aging is characterized by a progressive decline in biological functions. The molecular basis of aging mostly refer to the free radical theory of aging which postulates that reactive oxygen species (ROS) induce cellular damages leading to cell death. *S. cerevisiae* is a model organism to study chronological aging referring to the time period a yeast cell can survive in a non dividing state. It is measured by the loss of viability of stationary-phase cells. Viability in yeast is the cell ability to form colonies on Petri dishes. During stationary phase yeast cells evolve into two cell types: quiescent (Q) and non quiescent (NQ) cells. These two populations mainly differ by their ability to form colonies on Petri dishes, the Q cells being able and not the NQ cells. Moreover Q and NQ cells can be separated by differential centrifugation on density gradients (Allen *et al.* (2006) *J Cell Biol* **174**: 89–100). Global methods like proteomics allowed us to obtain an overall view of the effects of chronological aging on the proteome of yeast cell. We have compared the evolution of the yeast mitochondrial and cellular soluble proteomes using the Two-Dimensional Differential in-Gel Electrophoresis (2D-DIGE) technique at three times: 0 day (32 h after outset of yeast culture), 7 days and 14 days. The ratio Q/NQ cells is decreasing with time: 100% Q cells at day 0, 50% at day 7 and almost 0% at day 14. As during stationary phase yeast consumes ethanol it has produced during exponential phase on glucose we have followed ethanol and acetate concentrations until day 14. It appeared that yeast were under starvation at day 7. Then in order to discriminate changes linked to aging from those due to starvation we realized the same proteomics studies on cells kept at constant ethanol concentration during 14 days. Cellular and mitochondrial proteome analyses allowed us not only to follow proteomic adaptations occurring in cytosolic and mitochondrial compartments but also to get information about mitochondrial biogenesis by comparing the ratio of mitochondrial proteins found in both analyses.

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5P.4 Effects of caloric restriction, dietary restriction and every other day feeding on energy metabolism and redox state

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Calorie restriction (CR), or the limitation of ingested calories, but not micronutrients, is a well-established intervention that improves animal health and longevity. In a systemic review of the literature, we observed that in the last years, 60% of published papers used non-supplemented dietary restriction (DR) instead of CR. Every other day (EOD) feeding was used as an alternative to CR in 15% of published papers in this area. Little is known about the long-term health and longevity impact of DR and EOD diets, and we hypothesized that the lack of homogeneity in dietary protocols could account for diverging experimental results in the field. We submitted rats to 40% CR, 40% DR, or EOD feeding for 8 months. We found that EOD animals ingest equal total amounts of food than animals fed *ad libitum*, but presented significantly reduced body weight, similarly to CR and DR. The efficiency of energy conversion was decreased in EOD, CR and DR animals. Serological parameters were improved in the CR group, but not in DR or EOD diets. Respiration and H_2O_2 release in liver and brain were unaffected by dietary interventions, with the exception of brain in the CR group, which generated 50% less H_2O_2. O_2 consumption was reduced in adipose tissues in response to lower caloric intake, while muscle O_2 consumption was reduced in the groups which received no micronutrient supplementation. Most dietary interventions decreased H_2O_2 release in muscle and adipose tissue, with the exception of EOD animals. Levels of protein carbonyls and glutathione in all tissues were not affected by the restrictive diets. Malonaldehyde levels, were altered by the presence of micronutrients in the brain, and were unaffected in the other tissues. In muscle and adipose tissue, the diets increased catalase and SOD2 expression 2–3 fold. Catalase and glutathione peroxidase expression were increased in the brain with the dietary interventions. The expression of these enzymes was unaltered in the liver. Overall, our data indicates that CR presents the most prominent improvements in redox state and serological parameters. The lack of micronutrient supplementation in DR has a negative impact on animal health. EOD protocol presents significant differences in results compared to CR, and should not be used interchangeably. *Keywords*: caloric restriction, every other day feeding, dietary restriction, micronutrient supplementation, energy metabolism, redox state.

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5P.5 The role of the ubiquinone pool in modulating the superoxide production by the mitochondrial cytochrome bc_1 complex

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Production of reactive oxygen species (ROS) by the mitochondrial respiratory chain is considered to be one of the major causes of degenerative processes associated with oxidative stress. Mitochondrial ROS has also been shown to be involved in cellular signaling. It is generally assumed that the ubisemiquinone intermediate formed during turnover at the ubiquinol oxidation center (Q_o site) of the cytochrome bc_1 complex (complex III) is one of the two major sources of electrons for superoxide formation in mitochondria. We