for thermogenic needs of animals. Therefore thermogenesis on the level of brown-fat mitochondria and whole animal was examined here in PolgA mtDNA polymerase mutant mice (Mutator) exhibiting numerous mutations of mtDNA and several features of premature aging (Trifunovic et al., 2004). As compared with wild-type mitochondria, on all 3 substrates investigated (pyruvate, palmitoyl-L-carnitine and glycerol-3-phosphate), UCP1-dependent oxygen consumption was significantly reduced in mutant mitochondria similarly to maximal oxidative capacity (FCCP-response), indicating impaired thermogenesis on the level of brown-fat mitochondria in Mutator mice. Basal metabolic rate at 30 °C (thermoneutrality) was higher in mtDNA-Mutator mice as compared with WT mice; this may indicate changed set-point of the thermoregulatory centre. However, cold-induced metabolic rate (estimated as increase in oxygen consumption at 22 °C compared to 30 °C) in Mutator mice was only half of that in WT. At environmental temperatures below 20 °C, Mutator mice were unable to further increase their metabolism and went into torpor. Response to adrenergic stimulus (NE injection) was significantly reduced in Mutator mice. Thus, mtDNA mutation led to lower activity of brown-fat mitochondria and impaired thermogenesis; i.e. also in this respect, mtDNA-Mutator mice mimicked normal ageing.

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### S14.7 Impact of chronological aging on mitoproteome of *Saccharomyces cerevisiae*

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The free radical theory of aging postulates that reactive oxygen species (ROS) mainly produced by mitochondria are able to induce cellular damages leading to cell death. In *Saccharomyces cerevisiae*, chronological aging is related to the senescence over time of non dividing cells. In this work we study chronological aging of *S. cerevisiae* in stationary phase with a proteomic approach (Two Dimensional Differential in-gel electrophoresis methodology) in order to compare the mitochondrial proteome at three distinct periods (0 day, 7 days, and 14 days). Moreover, based on a recent study in stationary phase culture (Allen, 2006), we separated quiescent (Q) from non-quiescent (NQ) cells which mainly differ by their ability to form colonies on Petri dishes. Down-regulations of the major mitochondrial metabolic pathways (Krebs cycle, OXPHOS, amino-acid metabolism, protein synthesis, folding and import) were observed between 7 and 14 days. Interestingly, the only differential regulation observed between Q and NQ cells at 7 days is related to the ROS detoxifying enzyme glutathione transferase that was found to be more expressed in Q cells. This result suggests that Q cells mitochondria have a better capacity to resist to oxidative stress, and could partially explain why these latter cells are able to form colonies again.

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### S14.8 Top-down control analysis of mitochondrial oxidative phosphorylation: From mitochondria to pathologies

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The top-down approach of the metabolic control analysis, extensively used in our laboratory, has for example been applied on isolated mitochondria to describe the protective role of cyclosporin A on mitochondrial function during ischemia–reperfusion transitions. In this approach oxidative phosphorylation is described as large modules linked by a common intermediate. In this system the respiratory chain generates the proton-motive force, which is consumed by the phosphorylation subsystem and proton leak across the inner mitochondrial membrane. By monitoring simultaneously the kinetics of oxidation and phosphorylation rates and the membrane potential variations, it becomes possible to determine the elasticity of those three modules in response to small variations of the proton-motive force (obtained experimentally over a range of phosphorylation rates from state 4 to 3) and thus to access to the control scheme of oxidative phosphorylation. We will present our first results obtained on rat skeletal muscle mitochondria, in which respiratory chain exerts an important control, whatever the phosphorylation rate. This approach will be used in a near future to investigate the effect of aging and septic shock on mitochondrial function. With the top-down control analysis, we will seek to determine which modules or processes are affected by these conditions and thus better understand the very mechanisms responsible of observed mitochondrial and muscle dysfunctions.

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### S14.9 Testing the mitochondrial free radical theory of aging in *Drosophila melanogaster*

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Nowadays, the Mitochondrial Free Radical Theory of Aging (MFRTA) is the most supported theory to explain aging process. It is possible to deduce three predictions from MFRTA: 1) long-lived animals must produce fewer mitochondrial Reactive Oxygen Species (mtROS) than short-lived ones, 2) the decrease in mtROS generation must increase life span and 3) the increase in mtROS generation must decrease life span. In the present study we have used *Drosophila melanogaster* to test such predictions. First, we have study mtROS production in three different wild type strains of *Drosophila* (Dahomey, Canton-S and Oregon). According to MFRTA long-lived Oregon flies produce fewer mtROS than short-lived Dahomey or Canton-S. In order to test the second prediction we have introduce the alternative oxidase (AOX) gene from *Ciona intestinalis* in *Drosophila* genome. AOX expression decrease free radical production, but it does not increase mean or maximum life span at three different temperatures (18, 25 and 29 °C). We tested the third prediction in DJ-1 mutant flies. DJ-1 mutant flies produce significantly more free radicals than Oregon, Canton-S or Dahomey flies, however at 25 °C they do not live shorter than the longest-living background (Oregon). In summary, our results do not support MFRTA and they invite to re-think the role of mtROS in aging process.

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### S14.10 Estimation of membrane potential of rat liver mitochondrial particles by TMRE fluorescence in confocal mode

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The aim of this study was to estimate the membrane potential of isolated mitochondria on the single-particle level. We used a fluorescence correlation spectroscopy setup with a 532-nm laser to detect fluorescence signals of single TMRE-doped mitochondria in suspension. The brightness of the fluorescent particles increased after the addition of a respiratory substrate (succinate) in the presence of rotenone and decreased after the addition of an uncoupler (dinitrophenol). Thus, the fluorescence signals of the particles correlated well with membrane potential magnitudes under our experimental conditions. Using an empirical formula of Gaussian–Lorentzian distribution of the brightness in the confocal volume, we found the fluorescence intensity of a single energized mitochondrion passing through the center of the observation volume. Given the fluorescence intensity of a single TMRE molecule, we estimated the number of TMRE molecules bound to a single mitochondrial particle. The number of mitochondrial particles per mg of protein ($1.5 \times 10^9$) determined from the statistical distribution of fluorescence intensities and the magnitude of the membrane potential (190 mV) estimated by the Nernst equation were consistent with values of these parameters measured previously by other techniques.

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## (S15) $bc_1$ complexes symposium lecture abstracts

### S15/1 The $Q_O$ site semiquinone state in isolated cytochrome $bc_1$ (complex III) from *Rhodobacter capsulatus*

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The $Q_o$ site semiquinone of cytochrome $bc_1$ has been assigned pivotal roles in productive energy-conversion and destructive superoxide generation. After a 30 year search for direct evidence of this semiquinone state, a transient, $Q_o$ site inhibitor sensitive semiquinone EPR radical has been revealed in a genetic heme $b_H$ knockout, which effectively improves the energetics for semiquinone formation at the $Q_o$ site. This first observation was performed in native membranes of the purple photosynthetic bacterium *Rhodobacter capsulatus*. To remove possibilities that the signal was a result of either an unforeseen semiquinone state in another redox protein of the native membranes, or damage resulting from knockout of heme $b_H$, we have examined for the $Q_o$ site semiquinone state in isolated and purified cytochrome $bc_1$ equipped with a full complement of cofactors. Combined in a hybrid system with reaction centers (*Rba. sphaeroides*; thanks to Colin Wraight, Urbana-Champagne, IL), ubiquinone and cytochrome $c_2$ (*Rba. capsulatus* thanks to John Fitch and Michael Cusanovitch, Tucson, Az), light activation generates an EPR signal in a manner similar to that seen in native membranes and fully consistent with its identity as a key state of the cytochrome $bc_1$.

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### S15/2 Domain conformational switch of the iron sulfur protein in cytochrome $bc_1$ complex is induced by the electron transfer from cytochrome $b_L$ to $b_H$

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Intensive biochemical, biophysical and structural studies of the cytochrome (cyt) $bc_1$ complex in the past have led to the formulation of the "protonmotive Q-cycle" mechanism for electron and proton transfer in this vitally important complex. The key step of this mechanism is the separation of electrons during the oxidation of a substrate quinol at the $Q_P$ site with both electrons transferred simultaneously to ISP and cyt $b_L$ when the extrinsic domain of ISP (ISP-ED) is located at the *b*-position. Pre-steady state fast kinetic analysis of $bc_1$ demonstrates that the reduced ISP-ED moves to the $c_1$-position to reduce cyt $c_1$ only after the reduced cyt $b_L$ is oxidized by cyt $b_H$. Structural analyses of Pm or Pf inhibitor loaded crystals revealed two ISP-ED binding positions on cyt *b*. However, the question of how the conformational switch of ISP-ED is initiated remains unanswered. The results obtained from analysis of inhibitory efficacy and binding affinity of Pm and Pf inhibitors, under various redox states of the $bc_1$ complex, suggest that the electron transfer from heme $b_L$ to $b_H$ is the driving force for the releasing of the reduced ISP-ED from the *b* position to $c_1$ position to reduce cyt $c_1$.

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### S15/3 Regulatory interactions in the dimeric cytochrome $bc_1$ complex

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The dimeric cytochrome $bc_1$ complex catalyzes oxidation-reduction of quinol and quinone at sites located in opposite sides of the membrane in which it resides. The kinetics of electron transfer and inhibitor binding in the isolated yeast and *Pseudomonas denitrificans* $bc_1$ complexes reveal functional interactions between the quinol oxidation site at center P and quinone reduction site at center N in opposite monomers in conjunction with inter-monomer electron equilibration between the cytochrome *b* subunits of the dimer. The resilience of center P catalysis to inhibition caused by partial pre-reduction of the $b_H$ hemes can be explained by inter-monomer electron transfer between the two cytochrome *b* subunits in the $bc_1$ dimer. A model for the mechanism of the $bc_1$ complex has emerged in which binding of ligands that mimic semiquinone at center N regulates half-of-the-sites reactivity at center P and binding of ligands that mimic binding of ubiquinol at center P regulates half-of-the-sites reactivity at center N. An additional feature of this model is that inhibition of quinol oxidation at the quinone reduction site is avoided by allowing catalysis in only one monomer at a time, which maximizes the number of redox acceptor centers available in cytochrome *b* for electrons coming from quinol oxidation reactions at center P and minimizes the leakage of electrons that would result in the generation of damaging oxygen radicals.

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### S15/4 The loneliness of the electrons in the $bc_1$ complex

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