however, little is known about which proteins mediate ion transport across the mitochondrial inner membrane. Here, we uncover a novel mechanism regulating mitochondrial volume that depends on glutathione levels, the adenine nucleotide translocase, and substrate metabolism. Addition of 3 mM reduced (GSH), but not oxidized (GSSG; up to 0.3 mM), glutathione to isolated guinea pig heart mitochondria, in the absence of exogenous substrates, triggers a dramatic, switch-like, transient contraction and sustained swelling response involving matrix expansion and remodeling of inner membrane cristae. Remarkably, mitochondrial swelling could be reversed by tricarboxylic acid (TCA) cycle intermediates with a preferred selectivity of the substrate, as follows: citrate = isocitrate > succinate > malate > oxaloacetate > glutamate. Preincubation with the six TCA cycle intermediates either blunted or prevented the acute swelling response to 3 mM GSH depending on the substrate. The GSH-induced swelling occurred in parallel with acute NAD(P)H oxidation. Adding ADP and bongkrekic acid before GSH completely blocked the swelling response, or contracted mitochondria pre-swollen with GSH, indicating the adenine nucleotide translocase (ANT) was acting as a redox-sensitive pore. The response was insensitive to the permeability transition pore inhibitor cyclosporine A or the inner membrane anion channel inhibitor 4′-chlorodiazepam (4-Cl-DZP). The findings highlight an important interaction between the glutathione and pyrimidine nucleotide pools that participates in mitochondrial volume regulation by changing the conformation of the adenine nucleotide translocase.

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S14/3 Targeting molecules to mitochondria

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Mitochondrial function and dysfunction contributes to a range of important aspects of biomedical research. Consequently there is considerable interest in developing approaches to modify and report on mitochondria in cells and in vivo. One approach has been to target bioactive molecules to mitochondria by conjugating them to lipophilic cations. Due to the large mitochondrial membrane potential, the cations are accumulated within mitochondria inside cells. This approach had been used to develop mitochondria-targeted antioxidants that selectively block mitochondrial oxidative damage and prevent some types of cell death and also to develop probes of mitochondrial function. Here we outline some of the background to the development of these compounds.

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(S14) Mitochondria and ageing symposium abstracts (poster and raised abstracts)

S14.4 Random mtDNA mutations cause respiratory dysfunction through failure in complex assembly

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Mice with defective proofreading in the mitochondrial specific DNA polymerase polg, (mtDNA mutator mice) have increased mitochondrial DNA mutation rate and a premature aging phenotype, including hair loss, cardiomyopathy, early loss of fertility in females, anaemia, kyphosis, osteoporosis and progressive hearing loss. We set out to find out what effect the mutations would have on the mitochondria. Previously we have shown that there is a progressive loss of activity in respiratory chain complexes that are partially encoded by mtDNA (I, III, IV and V). We now show that isolated mitochondria from these mice display reduced oxidative capacity. Western blot analysis of single protein subunits shows that there is a reduction in levels of COX II and IV, while levels of all other analysed protein subunits appear to be normal. However, when we look at fully assembled complexes, using blue native electrophoresis, we observe a reduction in complexes I, III and IV. The detected reduction is not due to impaired mitochondrial translation as shown by in organello translation assays. We argue that point-mutations in the mitochondrial DNA alter the mitochondrialy encoded respiratory chain subunits leading to failed assembly and ultimately reduced oxidative capacity.

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S14.5 Does aging influence lymphocyte mitochondria respiration in trained people?

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One of the major explanations for age-related decrease in general functionality of cell tissues has been the decline in mitochondria functionality. However, it have been stated that exercise induces an increase in mitochondria functionality. So, the aim of this study was to analyze the influence of exercise training on lymphocyte mitochondria function with aging. Fifteen men, aged between 19 and 52 years old (average age = 32.6±10.6 years), engaged in regular physical activity participated in this study. Maximal aerobic capacity (VO_2max. ml kg^{-1} min^{-1}) was assessed by spirometry until exhaustion. Lymphocyte mitochondria oxidative activity of Complex I and Complex II were assessed. A Pearson correlation was performed in order to test variables (age, mitochondria complex I and II respiration rate) associations. Significance level was established at 5%. VO_2max was 55.66 (+6.23) ml kg^{-1} min^{-1} and mitochondrial oxidative rate was 12.9±5.5 nmol oxygen/min/mg protein and 19.9±8.1 nmol oxygen/min/mg protein for Complex I and Complex II, respectively. Our results couldn't find any significant correlation between mitochondria oxidative rates and age. Concerning the high values obtained in maximal aerobic capacity of this sample and the lack of correlation between mitochondria oxidative capacity and age, we may conclude about the positive effects of exercise training in mitochondria functionality opposing the effect of aging process.

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S14.6 Impaired thermogenesis in PolgA mtDNA polymerase mutant mice

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Impaired thermogenesis is one of the features of ageing. Activity and recruitment of mitochondria in brown adipose tissue is important

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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