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.

doi:10.1016/j.bbabio.2010.04.188

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

Arnaud Blomme[1], Allan Mac'Cord[1], Francis E. Sluse[1], Gregory Mathy[1,2]
[1]*Laboratory of Bioenergetics and Cellular Physiology*
[2]*Laboratory of Vegetal Biochemistry, Department of life Sciences, University of Liege, Belgium*
*E-mail:* ablomme@student.ulg.ac.be

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.

doi:10.1016/j.bbabio.2010.04.189

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

Fernanda M. Cerqueira, Fernanda M. Cunha, Camille C. Caldeira da Silva, Camila Carrião, Renato Lahos, Pio Colepicolo Neto, Alicia J. Kowaltowski
*Instituto de Química, Departamento de Bioquímica, Brazil*
*E-mail:* fernandamcerqueira@yahoo.com.br

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.

FAPESP, INCT Redoxoma.

doi:10.1016/j.bbabio.2010.04.190

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

Stefan Dröse, Ulrich Brandt
*Molecular Bioenergetics Group, Medical School, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany*
*E-mail:* droese@zbc.kgu.de

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

have shown that superoxide formation at the ubiquinol oxidation center of membrane-bound or purified cytochrome $bc_1$ complex is stimulated by the presence of oxidized ubiquinone when the ubiquinol reduction center ($Q_i$ site) is blocked [1]. This indicated that the electron is transferred onto oxygen from reduced cytochrome $b_L$ *via* ubiquinone in a reverse reaction rather than during the forward Q-cycle reaction. In intact rat heart mitochondria respiring on succinate, inhibitors (malonate, diazoxide, TTFA, and atpenin A5) of the succinate:ubiquinone oxidoreductase (complex II) stimulated mitochondrial ROS production at the $Q_0$ site of complex III under conditions of oxidant-induced reduction; this stimulation was greatly enhanced by uncoupling [2]. We conclude that cytochrome $bc_1$ complex linked ROS production is promoted by a partially oxidized rather than by a fully reduced ubiquinone pool. This mechanism of ROS production by complex III offers a straightforward rationale of how the redox state of the ubiquinone pool could play a central role in mitochondrial redox signaling.

**References**

[1] Dröse, Brandt (2008) *J. Biol. Chem.* **283:** 21649–21654.
[2] Dröse *et al.* (2009) *Biochim. Biophys. Acta* **1790:** 558–565.

doi:10.1016/j.bbabio.2010.04.191

### 5P.6 Role of conformational changes in mitochondrial complex I in the hypoxic response

Alexander Galkin[1], Nanci Frakich[2], Salvador Moncada[2]
[1]*Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL, UK*
[2]*The Wolfson Institute for Biomedical Research, University College London, London WC1E 6BT, UK*
*E-mail:* a.galkin@qub.ac.uk

Mitochondrial complex I plays a critical role in regulating cellular energy generation and the production of reactive oxygen species (ROS). Two catalytically and structurally distinct forms of mitochondrial complex I have been characterised in enzyme preparations *in vitro*: one is a fully catalytically competent, active (A)-form and the other is a dormant, silent or de-activated (D)-form. When deprived of substrate at physiological temperatures the idle enzyme undergoes conversion into the D-form. This can gradually convert back to the A-form in the presence of substrate (NADH and ubiquinone) during slow turnover(s) of the enzyme. In the D-form of complex I a critical cysteine-39 of the ND3 subunit becomes exposed to the outside of the enzyme and is susceptible to modification and inhibition by peroxynitrite, nitrosothiols or ROS. This cysteine group is not accessible to any covalent modification in the A-form. Using a cultured cell line we have shown that A-to-D transition occurred during anaerobic incubation, when the respiratory chain was reduced. Accumulation of the D-form of complex I may be protective because slow re-activation of the D-form may reduce the burst of damaging ROS that occurs after reoxygenation. We also demonstrated that re-activation of the D-form could be prevented by prolonged incubation with endogenously-generated nitric oxide (NO). It is possible, therefore, that in some circumstances NO-dependent formation of S-nitrosothiols or peroxynitrite may lead to modification of complex I when it is in its D-form and so impede its return to the active state. Indeed, accumulation of the covalently modified D-form is likely to be responsible for the so-called persistent inhibition of cellular respiration that occurs in the presence of NO. The detrimental effect of such irreversible locking of complex I in the D-form could be due to the fact that the modified D-form of the enzyme generates ROS at a higher rate than the A-form. Thus a combination of changes in mitochondrial ROS production, a change in NAD/NADH ratio and a decline in the rate of oxidative phosphorylation could lead to cellular death and might be responsible for ischaemic damage as well as for the early stages of neurodegeneration.

doi:10.1016/j.bbabio.2010.04.192

### 5P.7 The mechanism of metformin action in 3T3-L1 adipocytes

Andrea Anedda, Eunate Gallardo-Vara,
Eduardo Rial, Maria Mar González-Barroso
*Centro de Investigaciones Biológicas – CSIC, Madrid, Spain*
*E-mail:* margb@cib.csic.es

Metformin (dimethylbiguanidine) is the most commonly used drug for the treatment of type 2 non-insulin dependent diabetes mellitus. The beneficial effects of the drug include a decrease in blood glucose, without stimulating insulin secretion and a general improvement in peripheral insulin sensitivity. Additionally, treatment of type 2 diabetes with metformin is associated with an overall reduction in circulating lipids and body weight both leading to a lower cardiovascular risk. Metformin action involves AMPK activation although the metabolic consequences will vary in the different target tissues. In white adipose tissue metformin-induced AMPK activation stimulates catabolic pathways that results in the reduction of triglyceride stores as reflected by the smaller size of the adipocytes. Despite fifty years of research, the early steps of metformin action are far from being elucidated. It is known that biguanidine derivatives inhibit mitochondrial respiration and this effect is probably at the basis of their antidiabetic action. There are data suggesting an indirect effect of metformin and, thus, the existence of a cell-signalling pathway targeted to the respiratory chain has been proposed. The inhibition of respiration would be responsible of two important events that would signal or initiate a shift in the cellular metabolism: (1) a decrease in the ATP levels that could lead to AMPK activation and (2) an increase in the production of superoxide by the respiratory chain with the concomitant raise in the levels of reactive oxygen and nitrogen species. We have investigated the early effects of metformin on 3T3-L1 adipocytes. We have observed that metformin rapidly inhibits cellular respiration that leads to an increase in reactive oxygen species levels and to AMPK activation. UCP2 levels raise as part of the antioxidant response of the cell, since the presence of a superoxide scavenger blocks its induction. UCP2 mRNA levels are unchanged and therefore the raise in protein levels must reflect an increased mRNA translation. AMPK activation is rapid, is associated with the expected decrease in fatty acid synthesis and does not require either the induction of UCP2 or a drop in ATP levels. Interestingly, metformin inhibits pyruvate oxidation but does not prevent the oxidation of added fatty acids.

doi:10.1016/j.bbabio.2010.04.193

### 5P.8 Mitochondria-targeted antioxidants prevented ischemic injury of kidney

Stanislovas S. Jankauskas[1,2], Egor Y. Plotnikov[1], Irina B. Pevzner[1], Vladimir P. Skulachev[1], Dmitry B. Zorov[1]
[1]*A.N.Belozersky Institute of Physico-Chemical Biology and Institute of Mitoengineering, Moscow State University, Russia*
[2]*Russian State Medical University, Moscow, Russia*
*E-mail:* stanislovas@mail.ru