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.

FAPESP, INCT Redoxoma.

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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