various thiol agents, including the membrane permeable p-chloromercuribenzoate (pCMB) and *N*-ethylmaleimide, and the membrane impermeable p-chloromercuri-phenylsulfonic acid (pCMBS), were used to discriminate between the roles of the adenine nucleotide translocase (ANT) and the PiC in the molecular mechanism of the MPTP. We have investigated the effects of these agents added alone or together on MPTP opening, phosphate transport and the binding of the PiC and ANT to phenylarsine oxide (PAO) affinity column. A working model that accounts for our data will be presented.

Reference

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15P.24 A Ca^{2+}-regulated mitochondrial (permeability transition) pore in *Drosophila melanogaster*

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Mitochondria play a crucial regulatory role in cell death through opening of a high-conductance inner membrane channel with unknown structure, the mitochondrial permeability transition pore (PTP). Classical studies on the PTP were carried out mostly in mitochondria obtained from mammals. Although data are available also on yeast and plants, it is not clear whether the permeability changes can be ascribed to the same molecular events. Here, we have studied the properties of the PT in mitochondria from the fruit fly *Drosophila melanogaster*. We demonstrate Ca^{2+} uptake in *Drosophila* mitochondria, as well as a ruthenium red-insensitive Ca^{2+} release following matrix Ca^{2+} overload (which in mammals is caused by opening of the PTP). Ca^{2+} release was insensitive to CsA, Ub0 and ADP but was inhibited by Mg^{2+} (as is the PTP of all species) and Pi (as is the "pore" of yeast). Ca^{2+}-induced Ca^{2+} release in *Drosophila* mitochondria could be triggered by thiol reactive compounds such as mersalyl at low concentrations (20 μM) and N-ethylmaleimide at high concentrations (1-2 mM). Our results suggest that *Drosophila* mitochondria may possess a Ca^{2+}-regulated permeability pathway with features between the "pore" of yeast and the PTP of mammals.

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15P.25 Mitochondria quality-control: mechanisms involved in the downregulation of mitochondrial biogenesis by mitochondrial ROS in the yeast *Saccharomyces cerevisiae*

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Mitochondrial biogenesis is a complex process. It necessitates the participation of both the nuclear and the mitochondrial genomes. This process is highly regulated, and mitochondrial content within a cell varies according to energy demand. In yeast, there is now a growing amount of evidence showing that overactivation of the Ras/cAMP pathway leads to an increase in the cell mitochondrial content. The yeast *Saccharomyces cerevisiae* has three A kinase catalytic subunits, which are encoded by the TPK (*TPK1, TPK2*, and *TPK3*) genes. We show that, in the absence of the protein Tpk3p, mitochondria produce large amounts of reactive oxygen species (ROS) that signal to the nuclear transcription factors HAP2/3/4/5 (HAP complex) involved in mitochondrial biogenesis. We established that an increase in mitochondrial reactive oxygen species production down-regulates mitochondrial biogenesis. These results point to a role of ROS as signaling molecules in the cross-talk between mitochondria and nucleus. Furthermore, it is the first time that a redox sensitivity of the transcription factors involved in yeast mitochondrial biogenesis is shown. Such a process could be seen as a mitochondria quality control process. We further investigated the molecular mechanisms involved in the down regulation of mitochondrial biogenesis by ROS and were able to show that this originates in a decrease in the amount of Hap4p which is the co-activator of the HAP complex. This decrease is clearly linked to the mitochondrially generated oxidative stress and is reversed by both the addition of an antioxidant and the overexpression of the superoxide dismutase Sod1p. Further, because the heme molecule has always been considered as a possible regulator of the HAP complex and consequently of mitochondrial biogenesis, we investigated the effect of heme biosynthesis precursors (∂-aminolevulinate & deuteroporphyrin IX) on ROS-induced down regulation of mitochondrial biogenesis. We show that these precursors are able to regulate the Hap4p level in such a way that there is a reversion of mitochondrial biogenesis ROS-induced downregulation. Our study shows that mitochondrial biogenesis is downregulated by mitochondrial reactive oxygen species. This regulation goes through a modulation in the amount of the co-activator Hap4p and involves heme-induced regulation of this transcription factor.

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