15P.21 The Ca^{2+}-dependent ATP-Mg/Pi carriers as novel regulators of the mitochondrial permeability transition pore

Javier Traba[1], Ignacio Amigo[1], Carlos Rueda[1], Gyorgy Szabadkai[2], Michael R. Duchen[2], Jorgina Satrustegui[1], Araceli del Arco[3]
[1]*Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa UAM-CSIC, Universidad Autónoma, CIBER de Enfermedades Raras (CIBERER), Madrid, Spain*
[2]*Department of Cell and Developmental Biology, UCL London, UK*
[3]*Área de Bioquímica, Centro Regional de Investigaciones Biomédicas (CRIB), Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Toledo, Spain*
E-mail: jtraba@cbm.uam.es

The mitochondrial permeability transition pore (PTP) is a non-specific pore that is formed in the inner membrane under conditions of Ca^{2+} overload and/or oxidative stress[1,2,3]. The opening of the pore allows the entry of H^+, disrupts the mitochondrial DY, inhibits ATP synthesis and mitochondrial swelling occurs due to entry of water from the cytosol. The loss of cellular ATP causes a failure in ionic homeostasis and necrotic cell death. The molecular composition of the PTP is still unclear, but its implication in some types of cell death, as in situations of ischemia-reperfusion, has already been proved [1-3]. Adenine nucleotides are known to partially inhibit PTP opening in isolated mitochondria, by interacting with a high affinity site in the adenine nucleotide translocator. We have now identified the Ca^{2+}-dependent ATP-Mg/Pi carriers SCaMC-1/SLC25A24 and SCaMC-3/Slc25a23 as novel regulators of PTP opening in mammals. This carrier was functionally identified in the 80s by Aprille [4, 5], but the corresponding genes have been identified only very recently [6-10]. They regulate the mitochondrial concentration of adenine nucleotides in a Ca^{2+}-dependent way, and are able to regulate metabolic activities that have adenine nucleotide-dependent steps[4,5]. We have studied PTP opening in isolated mitochondria from mouse liver and brain, where SCaMC-3 is the main isoform, and in isolated mitochondria from cells lines, where SCaMC-1 is the main isoform. By using SCaMC-3 *knock-out* mice and SCaMC-1 stable *knock-down* cells, we have found that adenine nucleotides are able to regulate PTP opening independently of the adenine nucleotide translocator, in a way which depends on the presence of the ATP-Mg/Pi carrier. The modification of the mitochondrial adenine nucleotide content by the ATP-Mg/Pi carrier thus regulates the mitochondrial calcium retention capacity and PTP opening, and a major role for this novel mechanism of PTP regulation in cell death is proposed.

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15P.22 Different physiological uncoupling systems in yeast mitochondria

Salvador Uribe-Carvajal, Alfredo Cabrera-Orefice, Sergio Guerrero-Martínez, Manuel Gutiérrez-Aguilar, Luís Luévano-Martínez
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México
E-mail: suribe@ifc.unam.mx

In state IV isolated mitochondria consume O_2 at a slow rate. This is probably not observed *in vivo*: slow O_2 consumption would lead to accumulation of free radicals such as semiquinone. Thus, mitochondria must maintain high O_2 consumption. Yeast have evolved different uncoupling systems to ensure high O_2 consumption. We propose three such systems: the mitochondrial unspecific channel (MUC), the uncoupling protein and the alternative dehydrogenases. MUC is present in *S. cerevisiae* [1] and in *D. hansenii* [2]. It opens when ATP is not needed. Remarkably, depending on the environment where the yeast species normally lives, MUC is controlled by different effectors, e.g. in the sea yeast *D. hansenii* MUC is controlled by K^+ and/or Na^+. In *Y. lipolytica* [3] and in *D. hansenii* there are alternative oxido-reductases, namely a NADH dehydrogenase type 2 (NDH2) and alternative oxidase(s) (AOX). In *Y. lipolytica* growing in the log phase NDH2 is attached to a III/IV supercomplex, channeling its electrons to the proton pumping system. In contrast, in the stationary phase NDH2 is overproduced and found free. The free form is able to reduce AOX oxydizing NADH without pumping any protons, i.e. dissipating energy and maintaining high O_2 consumption. An uncoupling protein (UCP) was detected in *Y. lipolytica* [4]; UCP is overproduced in the stationary phase, resulting in high O_2 consumption. The existence of several energy dissipating systems in many yeast species is puzzling. These systems reflect the adaptability of mitochondria to the environment where the cell lives.

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15P.23 The mitochondrial permeability transition pore and its modulators

Pinadda Varanyuwatana, Andrew P. Halestrap
Department of Biochemistry, University of Bristol, UK
E-mail: p.varanyuwatana@bris.ac.uk

Previous data have provided strong evidence that the mitochondrial phosphate carrier (PiC) is the cyclophilin D binding component of the mitochondrial permeability transition pore (MPTP). In order to provide a more definitive proof, PiC knockdown by siRNA was carried out with subsequent determination of pore opening by calcium retention capacity assay. Our data showed some protection in cells where approximately 70% of the PiC (shown by western analysis) had been knockdown. However, we demonstrate that the calcium retention assay is inappropriate for studying the effects of PiC knockdown on MPTP opening because the lack of phosphate (Pi) decreases both mitochondrial membrane potential and the rate and extent of calcium uptake. We show that this may explain why Bernardi *et al.* [1] concluded that CsA inhibition of MPTP opening requires phosphate. Using a de-energised light scattering technique in the presence of a calcium ionophore we show that CsA inhibition of MPTP opening in liver mitochondria is independent of the presence of Pi. We will describe the development of similar techniques to measure MPTP opening in permeabilised cells subject to siRNA knockdown. We also report the results of experiments in which

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.

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

Sophia von Stockum[1], Emy Basso[1], Valeria Petronilli[1], Mike Forte[2], Paolo Bernardi[1]
[1]*Department of Biomedical Sciences and CNR Institute of Neurosciences, University of Padova, Italy*
[2]*Oregon Health and Sciences University, Portland, Oregon, USA*
E-mail: calvin-sophie@gmx.de

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*

Edgar Djaha Yoboue, Michel Rigoulet, Anne Devin
Institut de Biochimie et Génétique Cellulaires UMR5095 CNRS Université Bordeaux 2, 1 Rue Camille Saint-Saëns 33077 Bordeaux Cedex, France
E-mail: edgar.yoboue@ibgc.cnrs.fr

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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