

15P.21 The Ca²⁺-dependent ATP-Mg/Pi carriers as novel regulators of the mitochondrial permeability transition pore

Javier Traba¹, Ignacio Amigo¹, Carlos Rueda¹, Gyorgy Szabadkai², Michael R. Duchon², Jorgina Satrustegui¹, Araceli del Arco³

¹Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa UAM-CSIC, Universidad Autónoma, CIBER de Enfermedades Raras (CIBERER), Madrid, Spain

²Department of Cell and Developmental Biology, UCL London, UK

³Área 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 Ca²⁺ 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 Ca²⁺-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 Ca²⁺-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.

References

- [1] Leung, Halestrap (2008) *Biochim. Biophys. Acta* **1777**: 946-952.
- [2] Bernardi *et al.* (2006) *FEBS J.* **273**: 2077-2099.
- [3] Baines (2009) *J. Mol. Cell. Cardiol.* **46**: 850-857.
- [4] Aprille (1988) *FASEB J.* **2**: 2547-2556.
- [5] Aprille (1993) *J. Bioenerg. Biomembr.* **25**: 473-481.
- [6] del Arco, Satrustegui (2004) *J. Biol. Chem.* **279**, 24701-2413.
- [7] Fiermonte *et al.* (2004) *J. Biol. Chem.* **279**, 30722-30730.
- [8] Cavero, Traba, del Arco, Satrustegui (2005) *Biochem. J.* **392**: 537-544.
- [9] Traba, Froschauer, Wiesenberger, Satrustegui, del Arco (2008) *Mol. Microbiol.* **69**: 570-585.
- [10] Traba, Satrustegui, del Arco (2009) *Biochem. J.* **418**: 125-133.

doi:[10.1016/j.bbabbio.2010.04.386](https://doi.org/10.1016/j.bbabbio.2010.04.386)

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 O₂ at a slow rate. This is probably not observed *in vivo*: slow O₂ consumption would lead to accumulation of free radicals such as semiquinone. Thus, mitochondria must maintain high O₂ consumption. Yeast have evolved different uncoupling systems to ensure high O₂ 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 oxidizing NADH without pumping any protons, i.e. dissipating energy and maintaining high O₂ consumption. An uncoupling protein (UCP) was detected in *Y. lipolytica* [4]; UCP is overproduced in the stationary phase, resulting in high O₂ 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.

References

- [1] Gutiérrez-Aguilar M *et al.*, 2003, *Arch. Biochem. Biophys.* **494**: 184-191.
- [2] Cabrera-Orefice A *et al.*, 2010, *J. Bioenerg. Biomembr.* Electronic publication.
- [3] Guerrero-Castillo S *et al.*, 2009, *Biochim. Biophys. Acta-Bioenerg.* **1787**: 75-85.
- [4] Luévano-Martínez L *et al.*, 2010, *Biochim. Biophys. Acta* **1797**: 81-88.

doi:[10.1016/j.bbabbio.2010.04.387](https://doi.org/10.1016/j.bbabbio.2010.04.387)

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