

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

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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 SCA MC -1/SLC25A24 and SCA MC -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 SCA MC -3 is the main isoform, and in isolated mitochondria from cells lines, where SCA MC -1 is the main isoform. By using SCA MC -3 knock-out mice and SCA MC -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

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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 oxidizing 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

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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