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Review

The mitochondrial permeability transition from yeast to mammals

Luca Azzolin [a], Sophia von Stockum [a], Emy Basso [a], Valeria Petronilli [a], Michael A. Forte [b], Paolo Bernardi [a,*]

[a] Department of Biomedical Sciences, CNR Institute of Neuroscience, University of Padova, Italy
[b] Vollum Institute, Oregon Health and Sciences University, Portland, OR, USA

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ABSTRACT

Regulated permeability changes have been detected in mitochondria across species. We review here their key features, with the goal of assessing whether a "permeability transition" similar to that observed in higher eukaryotes is present in other species. The recent discoveries (i) that treatment with cyclosporin A (CsA) unmasks an inhibitory site for inorganic phosphate (Pi) [Basso, E., Petronilli, V., Forte, M.A. and Bernardi, P. (2008) Phosphate is essential for inhibition of the mitochondrial permeability transition pore by cyclosporin A and by cyclophilin D ablation. J. Biol. Chem. 283, 26307–26311], the classical inhibitor of the permeability transition of yeast and (ii) that under proper experimental conditions a matrix Ca^{2+}-dependence can be demonstrated in yeast as well [Yamada, A., Yamamoto, T., Yoshimura, Y., Gouda, S., Kawashima, S., Yamazaki, N., Yamashita, K., Kataoka, M., Nagata, T., Terada, H., Pfeiffer, D.R. and Shinohara Y. (2009) Ca^{2+}-induced permeability transition can be observed even in yeast mitochondria under optimized experimental conditions. Biochim. Biophys. Acta 1787, 1486–1491] suggest that the mitochondrial permeability transition has been conserved during evolution.

1. Introduction

The permeability transition (PT) is an increase of the inner mitochondrial membrane (IMM) permeability mediated by opening of the PT pore (PTP), a putative channel that has been thoroughly characterized functionally but whose molecular nature remains elusive [1]. Long considered an in vitro artifact of little pathophysiological relevance, the role of the PT in disease has been reevaluated in the context of both programmed and accidental cell death [2]. PTP openings of short duration lead to transient depolarization and to rearrangement of the cristae, making more cytochrome *c* available for release even in the absence of outer mitochondrial membrane (OMM) rupture provided that the Bax-Bak pathway had been activated [3]; while long-lasting openings cause permanent depolarization, loss of ionic homeostasis, depletion of matrix pyridine nucleotides, matrix swelling, OMM rupture and triggering of the mitochondrial pathway to apoptosis [4]. Under these conditions mitochondria hydrolyze any ATP available from glycolysis, and thus substantially contribute to energy depletion.

Mitochondrial swelling, its detrimental effects on energy conservation, and the basic features of the process (stimulation by Ca^{2+}, inorganic phosphate (Pi) and fatty acids and inhibition by Mg^{2+}, adenine nucleotides and acidic pH) have been recognized as soon as isolated mitochondria became available for biochemical studies [5–15]. These initial indications were reported before the chemiosmotic theory of energy conservation was proposed [16,17] and generally accepted [18]. How the chemiosmotic theory influenced studies of mitochondrial cation transport and of the PTP has been covered in some detail in previous reviews, to whom the interested Reader is referred for further details [2,19].

The subsequent history of the PTP can be traced to the work of Pfeiffer and co-workers, who proposed that it could play a role in steroidogenesis through a Ca^{2+}-dependent "transformation" of adrenal cortex mitochondria allowing extramitochondrial pyridine nucleotides to gain access to the otherwise impermeable matrix, in keeping with earlier observations [20], and support the 11-β hydroxylation of deoxycorticosterone [21–23]. Through the work of Haworth and Hunter, who coined the term "permeability transition", the basic features of the PTP in heart mitochondria were meticulously characterized, resulting in the key insight that the PT is due to reversible opening of a proteinaceous IMM pore [24–27]. The discovery that the PT can be desensitized by submicromolar concentrations of cyclosporin A (CsA) was a turning point [28–31] because it rekindled interest on the PTP, and provided a

Abbreviations: ANT, adenine nucleotide translocator; CsA, cyclosporin A; CyP, cyclophilin; IMM, inner mitochondrial membrane; NEM, N-ethylmaleimide; OMM, outer mitochondrial membrane; Pi, inorganic phosphate; PT, permeability transition; PTP, permeability transition pore; VDAC, voltage-dependent anion channel; YMUC, yeast mitochondrial unselective channel

* Corresponding author. Address: Department of Biomedical Sciences, University of Padova, Viale Giuseppe Colombo 3, I-35121 Padova, Italy. Fax: +39 049 827 6361.
E-mail address: bernardi@bio.unipd.it (P. Bernardi).

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pharmacological tool to address its role in cells and organs [32–37] as well as at the single channel level [38–40].

Most classical studies of the PT were carried out in mitochondria obtained from mammals, although permeability changes, most notably those caused by ATP and substrates, have also been studied in yeast [41–55]. In recent years, the growing interest on the PT in cell death has prompted an increasing number of studies in mitochondria from other organisms including plants [56–71], fish [72,73], amphibians [74,75], and the brine shrimp *Artemia franciscana*, a salt- and anoxia-tolerant organism that may represent an exception in that it apparently lacks a PT [76]. Whether the permeability changes observed in mitochondria from these organisms reflect the same molecular events underlying the PT of mammals is not obvious [46]. Here we compare the features of the PT in various organisms in the light of recent mechanistic advances of PTP regulation. We conclude that, with very few exceptions, regulated IMM permeability changes are a conserved feature of mitochondria across species.

2. Cyclophilin and the mechanism of PTP desensitization by cyclosporin A

CsA is a cyclic undecapeptide produced by the fungus *Tolypocladium inflatum*; its ability to prevent the immune response against xenografts [77] has allowed organ transplantation to become a standard surgical practice. This effect of CsA is mediated by two sequential events, (i) the interaction of CsA with cytosolic cyclophilin (CyP) A followed by the formation of a CsA·CyP-A complex; (ii) the binding of this complex to calcineurin, a Ca^{2+}/calmodulin-dependent cytosolic phosphatase that becomes inhibited [78–80]; as a consequence, phospho-NFAT is no longer dephosphorylated and therefore unable to translocate to the nucleus and trigger the IL-2-dependent activation of the immune response against the transplant [78–80].

CyPs are highly conserved, ubiquitous proteins sharing a common domain of about 109 amino acids, the CyP-like domain [81]; they possess peptidyl-prolyl *cis–trans* isomerase (PPIase) activity [82,83], which is inhibited after the binding of CsA [84]. Work with site-directed mutants of CyP-A has allowed the PPIase activity to be separated from CsA binding and calcineurin inhibition [85], and suggested that CyPs perform specific functions through interactions with a limited set of partner proteins rather than serving as general mediators of protein folding [81]. In keeping with this prediction, CyPs are involved in inflammation and vascular dysfunction [86–90], wound healing [91], innate immunity to HIV [92], hepatitis C infection [93], host-parasite interactions [94], tumor biology [95] and, in some species, regulation of the PTP which is mediated by CyP-D, the mitochondrial isoform of the enzyme [96–99]. Genetic ablation of the mouse *Ppif* gene (which encodes for CyP-D) has demonstrated that CyP-D is a unique mitochondrial receptor for CsA, and that it is responsible for modulation of the PTP but not a structural pore component [100–103] (see [104] for a recent review on the pathophysiology of CyP-D).

An important point should be appreciated, i.e. that CsA is *not* a *bona fide* PTP blocker. The inhibitory effect of CsA (and of CyP-D ablation) on the PTP is best described as "desensitization" in that the PTP becomes more resistant to opening after the uptake of Ca^{2+} and Pi in isolated mitochondria, yet opening still takes place for Ca^{2+}–Pi loads that are about twice those required in naïve mitochondria [2]. The mechanism through which CyP-D modulates the PTP has been recently clarified with our discovery that CyP-D ablation (or treatment with CsA) unmasks an inhibitory site for Pi, which is the actual PTP desensitizing agent [105]. It is remarkable that the PT of *Saccharomyces cerevisiae* mitochondria, which is insensitive to CsA in spite of the presence of a mitochondrial CyP, is inhibited by Pi [44]. As should become clear later in the review, we believe that inhibition by Pi is the unifying feature of the PT in all organisms, which allows to fill the gap between "CsA-sensitive" and "CsA-insensitive" PT, and to shed new light on what we regard as an evolutionarily conserved event.

3. The permeability transition in mammals

The PT has been characterized in a large number of tissues, cells and mitochondria of mammalian origin; due to space constraints, here we will only cover the essential regulatory features that allow a comparison to be made with other organisms, while we refer the Reader to previous reviews for a discussion of the pathophysiology of the PTP and its role in cell death [1,2,19,106–113].

Matrix Ca^{2+} is perhaps the single most important factor required for PTP opening. It is difficult to separate the PTP-inducing effects of Ca^{2+} from those of Pi (and possibly of polyphosphate generated in the matrix [114,115]), because the transport of Pi (or of other species able to prevent the buildup of a relevant ΔpH such as acetate or bicarbonate, see discussion in [105]) is required for the uptake of substantial amounts of Ca^{2+}. As discussed in detail elsewhere, matrix Ca^{2+} is best viewed as a permissive factor for PTP opening [2] in the sense that a PT is not observed in the absence of matrix Ca^{2+}, yet Ca^{2+} alone is not sufficient to induce PTP opening. The effect of Ca^{2+} is counteracted by other Me^{2+} ions that are transported by the Ca^{2+} uniporter (such as Sr^{2+} and Mn^{2+}), but the matrix Me^{2+} binding site(s) remain undefined. All divalent cations, including Mg^{2+} and Ca^{2+} itself, instead favor PTP closure through an external Me^{2+} binding site [116]. In the absence of a Ca^{2+} uniporter allowing fast Ca^{2+} uptake in energized mitochondria, it has been hard to assess the importance of matrix Ca^{2+} in *S. cerevisiae*, but recent work with the Ca^{2+} ionophore ETH129 and of proper concentrations of Pi has recently allowed to establish a Ca^{2+}-dependence in this organism as well [52] (see the following paragraph).

As mentioned above, CsA desensitizes the PTP of mammalian mitochondria through an effect on matrix CyP-D. Binding of CsA to CyP-D unmasks an inhibitory site for Pi, which is the actual PTP antagonist [105]. At variance from what is often, and erroneously, stated CsA is not a blocker of the PTP, and a PT can readily occur in the absence of CyP-D and in CsA-treated mitochondria and cells. Another important feature of the mammalian PTP is its modulation by redox effectors, a more oxidized state favoring PTP opening. This effect is conferred by at least three redox-sensitive sites, i.e. (i) a matrix SH site in apparent redox equilibrium with glutathione, which can be blocked by low micromolar concentrations of N-ethylmaleimide (NEM) and monobromobimane but not by impermeant bimanes or β-hydroxybutyrate; (ii) a matrix site in apparent redox equilibrium with pyridine nucleotides, which can be blocked by NEM or β-hydroxybutyrate but not by monobromobimane [117,118]; and (iii) an external thiol that triggers PTP opening after reaction with millimolar concentrations of NEM or low micromolar concentrations of copper-*o*-phenantroline [119]. This complex array of PTP redox-sensitive sites has recently been confirmed by careful studies based on mitochondrial photoirradiation after loading with hematoporphyrin [120].

4. The permeability transition in yeast

Manon and co-workers have published a very useful review of the properties of the "yeast PTP", also called yeast mitochondrial unselective channel (YMUC), and effectively summarized earlier literature on yeast permeability pathways [46]. They have also compared the features of the PTP of yeast and mammals as of 1998, and concluded that even if YMUC presents some functional analogies with mammalian PTP, its regulation is different enough