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Review

Mitochondrial permeability transitions: how many doors to the house?

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Abstract

The inner mitochondrial membrane is famously impermeable to solutes not provided with a specific carrier. When this impermeability is lost, either in a developmental context or under stress, the consequences for the cell can be far-reaching. Permeabilization of isolated mitochondria, studied since the early days of the field, is often discussed as if it were a biochemically well-defined phenomenon, occurring by a unique mechanism. On the contrary, evidence has been accumulating that it may be the common outcome of several distinct processes, involving different proteins or protein complexes, depending on circumstances. A clear definition of this putative variety is a prerequisite for an understanding of mitochondrial permeabilization within cells, of its roles in the life of organisms, and of the possibilities for pharmacological intervention.

Keywords: Permeability transition; Adenine nucleotide transporter; Bax; Cyclosporin A; Protein import; Mitochondria

1. Introduction

The mitochondrial permeability transition (PT) (reviews: Refs. [1–8]) consists in the opening of a permeation pathway allowing the diffusion of solutes up to about 1500 Da from the mitochondrial matrix to the extramitochondrial space, and vice versa. The phenomenon, characterized in landmark studies by Hunter and Haworth in the late 1970s [9–11], has recently attracted renewed attention because of its proposed roles in the release of cytochrome *c* and other pro-apoptotic factors in many models of apoptosis (reviews: Refs. [7,12–17]) including ischemia/reperfusion-induced tissue damage (reviews: Refs. [8,18–27]). The permeability transition is a complicated process, with many recognized inducers, modulators and inhibitors, some of which are mentioned below. Its most evident characteristic is a requirement for Ca^{2+} accumulation in the mitochondrial matrix (but see discussion below). In most cases, the PT has been studied using isolated mitochondria (particularly rat liver) and methods (mitochondrial swelling, depolarization, Ca^{2+} release) that can report on the opening and operation of the "pore" (PTP), but are not well suited to provide detailed information on the nature and properties of this pathway. The same can be said of the techniques used for the detection and characterization of the PT within intact cells or in tissues, which involve tracking the release from or entrapment in mitochondria of Δψ-indicating dyes or of PTP-permeant molecules [24,28–33]. These technical limitations may have hampered full appreciation of the long suspected (e.g.: Refs. [2,34]) multiplicity of biochemical species and processes which may lead to non-lytic permeabilization of the inner mitochondrial membrane. Here we present a brief review of the evidence suggesting such a multiplicity, with particular attention to the hypothesis that one of the forms of the

Abbreviations: ANT, adenine nucleotide translocator; CATR, Carboxyatractyloside; CK, creatine kinase; CSA, cyclosporin A; CypD, cyclophilin D; GSK-3β, glycogen synthase kinase 3β; HK, hexokinase; MCC, multiple conductance channel; MMC, mitochondrial megachannel; PA_2, phospholipase A_2; PhAsO, PhenylArsineOxide; PT, permeability transition; PTP, permeability transition pore; PK, protein kinase; ROS, reactive oxygen species; TFP, Trifluoroperazine; VDAC, voltage-dependent anion channel (mitochondrial porin)

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permeability transition pore may correspond to (a) complex(es) of the protein import machinery.

2. The PTP as ANT-centered complex(es)

Current ideas about the molecular nature of the PTP are varied, perhaps reflecting reality. For a period it was thought to be a membrane "defect" due to the production of lysophospholipids by a Ca^{2+}-dependent mitochondrial phospholipase A_2 [1,2]. This model has now been largely abandoned (but see below), but it remains true that both Ca^{2+}-dependent [35] and -independent [36] PA_2s are present in mitochondria, and produce free fatty acids which are powerful co-activators of the Ca^{2+}-induced PT [37,38]. Due largely to the discovery of a potent PT inhibitor which does not affect PA_2 activity, Cyclosporin A (CSA), and to the identification of the permeability transition pore with a gating channel observed by patch clamp, today the PTP is generally believed to be formed by proteins.

Since potential PTP precursor complexes can be readily isolated by biochemical means, they must be relatively abundant (and stable), although a quantitative estimate is difficult. Their transition to the unselective pore is relatively difficult: even under very favourable experimental conditions, in vitro, the PT takes several seconds to spread through a population of even the most susceptible mitochondria. In patch-clamp experiments, the pore thought to correspond to the PTP rarely appears in more than one copy per patch. This is of course to be expected for a process which needs to be tightly controlled and which requires only one or a few pores to open to disable a mitochondrion. The number of open pores per mitochondrion may, however, increase with increasing inducer concentration [39].

What proteins form the PTP is still open to question. A widespread consensus model envisions a supramolecular complex spanning the double membrane system of mitochondria, localized at contact sites [7,40–42]. Components of all mitochondrial compartments have been proposed to participate (e.g.: Refs. [43,44]; reviews: Refs. [8,45]). While the mitochondrial *cis–trans* peptidyl-prolyl isomerase Cyclophilin D (CypD) (matrix), the adenine nucleotide translocator (ANT) (inner membrane) and porin (VDAC) [46–49] presumably form the core of the complex (reviews: Refs. [8,50]), creatine kinase [46,51,52] (periplasmic space), the peripheral benzodiazepine receptor [53–57] (outer membrane) and VDAC-associated hexokinase (cytoplasm) [46,52,58–61] are also thought to have roles. The pro-apoptotic Bcl-2 family protein Bax might be part of the assembly (see below) and other proteins such as the BH3-only Bcl-2 family protein NOXA [62], anti-apoptotic members of the same family [63–65], and kinases such as PKA [66], PKG [67], a PKC isoform [68] and GSK-3β [69] may have roles as regulators.

For some of these putative components, data exist which suggest their presence may be optional or depend on tissue or circumstances, and that, therefore, a variety of permeability transition pores may exist. The PTP-forming complexes probably are transient, capable of disassembling and reassembling—perhaps with a different composition—with a relatively high frequency and/or in response to appropriate stimuli. Whether VDAC itself is a necessary component is debated [8]. The contact sites comprising ANT and VDAC are known to be under metabolic control, i.e., to be reversible [70–72]. A key component of the PTP, Cyclophilin D, is a matrix protein, although it can bind to the ANT (e.g.: Ref. [73]). This implies the existence of a dynamic association/dissociation equilibrium. It has been suggested that ANT-VDAC interaction may depend on CypD binding to the former [41]. Association of hexokinase (HK) to mitochondrial VDAC is known to depend on the cytoplasmic concentration of Glucose-6-phosphate [74–76]. It has been proposed, on the basis of biochemical (complex isolation) data, that HK may bind to a particular conformation of VDAC, induced by interaction between VDAC and the ANT in its "c" (atractyloside-stabilized) conformation (ANT1 was the isoform in the relevant experiments) [46,61]. HK association would be in competition with the formation of complexes between the ANT, octameric mitochondrial creatine kinase (mtCK), and VDAC. mtCK, located in the periplasmic space and interposed between ANT and VDAC, would act to prevent the PT (provided creatine is present) [46,51,77]. This type of interplay would be tissue-specific, since CK is not expressed in mammalian liver.

Let us now consider some of the major components of the "classical" PTP complex, the ANT, CypD and Bax.

2.1. ANT

Adenine nucleotide translocator ligands can induce (atractyloside) or repress (bongkrekate, adenine nucleotides) the PT (e.g: Refs. [78,79]). For these and other good reasons, many researchers have long considered the ANT (presumably as a dimer) to form the PTP, or to be the centerpiece of the complex forming it (reviews: Refs. [5,8,80]). The recently published structure of the ANT–CATR complex [81] is broadly compatible with the detailed model developed mainly by Halestrap's group [8]. For example, ANT Pro61, thought to be essential for CypD binding and action, is indeed appropriately located in a matrix-exposed surface helix. Purified ANT can be converted by Ca^{2+} into a high-conductance channel [82–84] bearing some similarity to channels observed by patch-clamping mitoplasts and assigned to the PTP (see below). The reconstituted Pi carrier can also form channels [85]—although with characteristics quite different from those expected for the PTP—and so might other transporters as well [86,87]. A recent paper has shown that a Ca^{2+}-induced PT takes place also in the mitochondria of mouse liver lacking both ANT genes [88]. Modestly (threefold) higher loads of Ca^{2+} were required to induce swelling of the mutant