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Review

Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore

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ABSTRACT

The mitochondrial permeability transition pore (MPTP) plays a key role in cell death, especially necrosis, and mediates the injury tissues such as the heart and brain experience following ischaemia and reperfusion. However, the molecular identity of the MPTP remains uncertain. Knockout studies have confirmed a role for cyclophilin-D (CyP-D) in pore opening, probably mediated by its peptidyl–prolyl *cis–trans* isomerase activity that facilitates a conformational change in an inner membrane protein. However, similar knockout studies have cast doubt on the central role of the adenine nucleotide translocase (ANT), previously regarded as a leading contender for the membrane component that forms the transmembrane channel of the MPTP. Here we review the evidence for and against a role for the ANT in MPTP opening and conclude that it usually plays a regulatory role rather than provide the transmembrane pore component. We suggest that the protein fulfilling the latter role is the mitochondrial phosphate carrier (PiC) and summarise recent evidence in support of this proposal. Our data are consistent with a model for the MPTP in which a calcium-triggered conformational change of the PiC, facilitated by CyP-D, induces pore opening. We propose that this is enhanced by an association of the PiC with the "c" conformation of the ANT. Agents that modulate pore opening may act on either or both the PiC and the ANT.

1. Introduction

The mitochondrial permeability transition pore (MPTP) is a non-specific pore, permeable to all molecules of less than 1.5 kDa, which opens in the inner mitochondrial membrane (IMM) under conditions of calcium overload. Opening is greatly enhanced by adenine nucleotide depletion, elevated phosphate and oxidative stress. These are conditions known to accompany reperfusion following a period of ischaemia, and in response to others stresses such as the application of certain metabolic poisons and toxins. Indeed the opening of the MPTP is now recognised to be a major cause of the necrotic cell death occurring under such conditions (see [1–4]). The role, if any, of the MPTP in healthy cells remains unclear since mice lacking cyclophilin-D, a component of the MPTP, show know obvious phenotype other than being protected against ischaemic injury [5–8].

1.1. The role of the MPTP in necrotic cell death

The role of the MPTP in necrotic cell death can be readily explained. Once the pore opens it allows free passage of protons across the inner membrane leading to a dissipation of the membrane potential and pH gradient that comprise the proton motive force. Not only does this prevent ATP generation by oxidative phosphorylation, but reversal of the ATPase occurs causing the breakdown of cytosolic ATP generated by glycolysis. As a result tissue ATP levels become severely compromised and, left unchecked, these will lead to major perturbations in the ionic and metabolic homeostasis of the cell. Ultimately these changes will cause necrotic cell death through the activation of phospholipases, nucleases and proteases [4,9,10]. Perhaps the best documented example of the role of the MPTP in necrotic cell death is in reperfusion injury of the heart [1,9], liver [11] and brain [12,13]. Here, the ischaemic phase of the insult causes calcium concentrations to rise and the production of some reactive oxygen species (ROS) that leads to oxidative stress. Although ROS and calcium are potential triggers of MPTP opening, the enhanced glycolysis that occurs during ischaemia leads to accumulation of lactic acid and a decrease in intracellular pH. This prevents MPTP opening which is progressively inhibited as the pH drops below 7 [14]. However, upon reperfusion there is a burst of ROS formation and the pH returns to normal, stimulating pore opening and hence cell death [1,9].

The importance of the MPTP in the necrotic death of the heart, brain and liver under such conditions was initially recognised through the

Abbreviations: ANT, adenine nucleotide translocase; BKA, bongkrekic acid; CAT, carboxyatractyloside; CsA, cyclosporin A; CyP, cyclophilin; IMM, inner mitochondrial membrane; MPTP, mitochondrial permeability transition pore; NEM, *N*-ethylmaleimide; PAO, phenylarsine oxide; PiC, mitochondrial phosphate carrier; PPIase, peptidyl–prolyl *cis–trans* isomerase; ROS, reactive oxygen species; SfA, sanglifehrin A; VDAC, voltage dependent anion channel

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use of MPTP inhibitors such as cyclosporin A (CsA) and sanglifehrin A (SfA) [15–18]. Furthermore, it is the hippocampus of the brain that is most vulnerable to ischaemic and hypoglycaemic damage, and mitochondria from this region are more susceptible to MPTP opening in vitro than are mitochondria from the cortex or cerebellum [12,13,19]. Most recently, further proof of a critical role for MPTP opening in necrotic cell death has been provided by the use of mice in which the target of CsA and SfA, cyclophilin-D (CyP-D — see below) had been knocked out. These animals showed substantial protection from ischaemia/reperfusion damage (infarct size) of both the heart [6,7] and brain [8]. In addition, the use of these mice has shown that cardiac failure associated with chronic calcium overload also involves MPTP-dependent death of cardiac myocytes [20].

1.2. The role of the MPTP in apoptosis

Another consequence of MPTP opening is swelling of the mitochondrial matrix, and this may play a role in apoptotic cell death under some conditions. Swelling occurs because the MPTP is permeable to all solutes of <1.5 kDa and once open the non-protein components of the mitochondrial matrix will rapidly equilibrate across the IMM. However, the matrix proteins remain and because they are at a higher concentration than in the cytosol, they will exert a colloidal osmotic pressure. It is this that causes the matrix to swell, the folds of the cristae allowing this to occur without breaking the IMM. However, the outer membrane cannot accommodate much swelling of the matrix without the outer membrane rupturing. Once such rupture occurs the contents of the inter membrane space are released including cytochrome *c* and other pro-apoptotic proteins such as Smac/Diablo and Apoptosis Inducing Factor (AIF). Thus, even if MPTP opening is insufficient to deplete ATP levels and cause necrosis, apoptosis may result [4,4,10,21]. It should be noted, however, that release of pro-apoptotic proteins from the inter membrane space is normally mediated by specific permeabilisation of the outer membrane through the action of pro-apoptotic members of the Bcl-2 family such as Bax [22,23].

2. The molecular mechanism of the MPTP

In view of its critical role in cell death, it is clearly important to understand the molecular mechanism of the MPTP. Several models have been proposed and although there remains no consensus as to the exact mechanism, there is increasing certainty that cyclophilin-D (CyP-D) and the adenine nucleotide translocase (ANT) play important roles. The remainder of this review will summarise the evidence for their involvement and provide evidence that additional components remain to be identified. We will briefly outline evidence from our laboratory that one such component may be the mitochondrial phosphate carrier (PiC).

2.1. The role of cyclophilin-D

A key to elucidating the mechanism of the MPTP was provided by Crompton and colleagues who first reported that sub-micromolar concentrations of cyclosporin A (CsA) inhibit pore opening [24]. Studies from this laboratory revealed that the potency of several CsA analogues as inhibitors of the pore correlated with their ability to inhibit the activity of a matrix peptidyl–prolyl *cis–trans* isomerase (PPIase) [25,26]. We subsequently purified this PPIase and identified it as cyclophilin-D (CyP-D) [27]. CyP-D is a protein encoded by the *PPIF* gene of the nuclear genome. It is synthesised in the cytosol and enters the mitochondria using a mitochondrial targeting sequence which is subsequently cleaved off [28,29]. The importance of CyP-D has recently been confirmed by the demonstration that mitochondria from CyP-D knockout mice do not exhibit CsA-sensitive MPTP opening [6,7,30]. However, it is important to note that MPTP opening can still be demonstrated in these mitochondria if the calcium loading of the mitochondria is increased sufficiently. This is also the case for wild-type mitochondria that have been treated with CsA [31]. These data support the hypothesis that pore opening involves a conformational change in a membrane protein that is facilitated by the PPIase activity of CyP-D but that can occur in the absence of CyP-D at higher [Ca^{2+}].

Cyclosporin A has some disadvantages as an inhibitor of CyP-D since it forms a complex with cytosolic cyclophilin-A (CyP-A) that inhibits the calcium activated protein phosphatase calcineurin. Indeed, it is through this pathway that the drug mediates its immunosuppressive effects [32]. Thus when used in vivo CsA has the potential to exert many other effects on cellular function independent of its inhibition of MPTP opening (for examples see [33,34]). Consequently, several CsA analogues have been developed that lack the ability to inhibit calcineurin but which are still active as inhibitors of the PPIase activity of CyP-D. These include 6-methyl-ala-CsA, 4-methyl-val-CsA (NIM-811) and 3-D-methyl-ala-4-ethyl-val-CsA (DEBIO-025) [35–37]. In addition we have shown that an unrelated immunosuppressant, sanglifehrin A (SfA), is also a potent inhibitor of the PPIase activity of CyP-D and inhibits the MPTP whilst being inactive against calcineurin, [18]. However, SfA shows some differences from CsA in the way that it inhibits MPTP opening. The concentration dependence of MPTP inhibition by SfA is sigmoidal unlike CsA which shows a linear relationship [18]. Furthermore, SfA does not prevent CyP-D binding to the inner membrane component of the MPTP whilst CsA inhibits binding. Thus it would seem that SfA acts to inhibit MPTP opening by inhibiting the conformation change catalysed by the bound CyP-D rather than by CyP-D binding [18].

The data reviewed above established beyond doubt the role of CyP-D in facilitating the opening of the MPTP. However, the identity of the membrane protein that binds CyP-D is less certain. Indeed it is possible that there is no specific protein involved and such a view underlies the model for MPTP formation proposed by He and Lemasters [38]. In this model it is proposed that the MPTP forms as a result of the aggregation of misfolded integral membrane proteins that have been damaged by oxidant and other stresses. CyP-D will normally block conductance through these protein aggregates, but when protein clusters exceed the CyP-D available to block conductance, unregulated pore opening occurs that is stimulated by calcium and inhibited by CsA binding to CyP-D. The apparent involvement of the adenine nucleotide translocase (ANT) in pore formation (see below) is explained in terms of the high amount of this protein in the inner mitochondrial membrane and its susceptibility to oxidative damage. Thus it is the protein most likely to form aggregates. However, as outlined below, the activation and inhibition of MPTP opening by different ligands of the ANT that cause opposite changes in its conformational state argues against such a non-specific effect.

2.2. The adenine nucleotide translocase

2.2.1. Evidence for a role of the ANT in MPTP formation

The most widely accepted candidate for the membrane component of the MPTP, first proposed by us in 1990 [25], is the adenine nucleotide translocase (ANT). The evidence for its involvement is considerable; it is reviewed extensively elsewhere [39] and will only be summarised here. Early observations from several laboratories, including our own, showed that MPTP opening is enhanced by adenine nucleotide depletion and inhibited by addition of ATP or ADP. Opening is also modulated by other specific ligands of the ANT including carboxyatractyloside (CAT) and bongkrekic acid (BKA). CAT induces the "c" conformation of the ANT and sensitises pore opening to [Ca^{2+}]. Conversely, BKA enhances the "m" conformation" and inhibits pore opening [25,31,40,41]. In addition, the ability of nucleotides to inhibit pore opening correlates with their ability to act as transportable substrates for the ANT [31].

More direct evidence that the ANT might bind CyP-D in a CsA-sensitive manner was provided through the use of a CyP-D affinity