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Role of the mitochondrial membrane permeability transition in cell death

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Abstract In recent years, the role of the mitochondria in both apoptotic and necrotic cell death has received considerable attention. An increase of mitochondrial membrane permeability is one of the key events in apoptotic or necrotic death, although the details of the mechanism involved remain to be elucidated. The mitochondrial membrane permeability transition (MPT) is a Ca^{2+}-dependent increase of mitochondrial membrane permeability that leads to loss of $\Delta\psi$, mitochondrial swelling, and rupture of the outer mitochondrial membrane. The MPT is thought to occur after the opening of a channel that is known as the permeability transition pore (PTP), which putatively consists of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocator (ANT), cyclophilin D (Cyp D: a mitochondrial peptidyl prolyl-*cis*, *trans*-isomerase), and other molecule(s). Recently, significant progress has been made by studies performed with mice lacking Cyp D at several laboratories, which have convincingly demonstrated that Cyp D is essential for the MPT to occur and that the Cyp D-dependent MPT regulates some forms of necrotic, but not apoptotic, cell death. Cyp D-deficient mice have also been used to show that the Cyp D-dependent MPT plays a crucial role in ischemia/reperfusion injury. The anti-apoptotic proteins Bcl-2 and Bcl-x_L have the ability to block the MPT, and can therefore block MPT-dependent necrosis in addition to their well-established ability to inhibit apoptosis.

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Introduction

Apoptosis is a form of programmed cell death and an outline of the relevant signaling pathways at the molecular level is now well established. Mammalian cells possess two major apoptotic signaling pathways, which are known as the intrinsic pathway and the extrinsic pathway [1]. The intrinsic pathway involves an increase of outer mitochondrial membrane permeability that leads to the release of various proteins from the intermembrane space into the cytoplasm, including apoptogenic molecules such as cytochrome *c*, Smac/Diablo, HtrA2 (Omi), AIF, and DNaseG [1, 2]. In the presence of ATP (dATP), cytochrome *c* binds to Apaf-1 and triggers its oligomerization, after which pro-caspase-9 is recruited and undergoes autoactivation. The protein complex comprising cytochrome *c*, Apaf-1, and caspase-9 is called the "apoptosome". In short, an increase of outer mitochondrial membrane permeability is central to apoptosis [3, 4], and mitochondrial membrane permeability is directly regulated by the Bcl-2 family of proteins [4, 5] (see Fig. 1). However, the detailed mechanisms underlying the increase of outer mitochondrial membrane permeability during apoptosis and how this process is controlled by Bc1-2 family members are still to be determined. The model that was initially developed to explain the apoptotic increase of mitochondrial membrane permeability was based on the "mitochondrial membrane permeability transition" (MPT) [6], an event which has been appreciated for some time among investigators studying the mitochondria. This review summarizes recent progress with

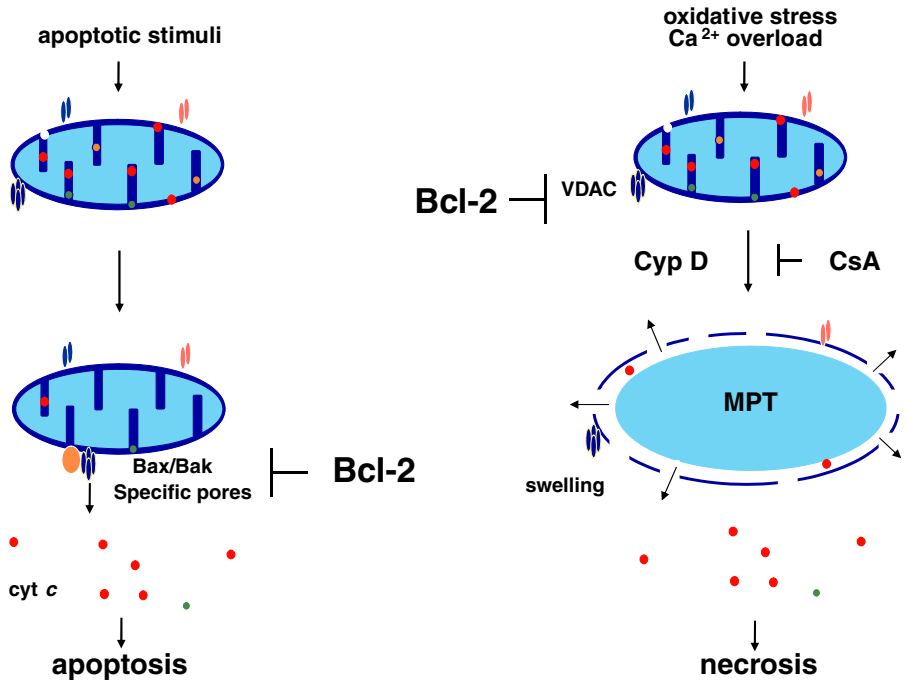

Fig. 1 Role of the mitochondria in apoptosis and necrosis. An increase in the permeability of the outer mitochondrial membrane is crucial for apoptosis to occur and is regulated by multidomain pro-apoptotic members of the Bcl-2 family (Bax and Bak), resulting in the release of several apoptogenic factors into the cytoplasm. In contrast, the Cyp D-dependent MPT involves an increase in the permeability of both the outer and inner mitochondrial membranes, and leads to necrosis induced by Ca^{2+} overload and oxidative stress. Both types of mitochondrial membrane permeability change are inhibited by anti-apoptotic members of the Bcl-2 family (Bcl-2 and Bcl-x_L)

regard to our understanding of the role of the MPT in cell death.

MPT

Mitochondria isolated from a variety of sources can show a sudden increase in the permeability of the inner mitochondrial membrane to solutes with a molecular mass of less than 1,500 Da, which results in the loss of $\Delta\psi$, mitochondrial swelling, and rupture of the outer mitochondrial membrane [7, 8] (see Fig. 1). This process is called the mitochondrial membrane permeability transition (MPT). The MPT can be induced under various conditions, such as exposure of mitochondria to Ca^{2+} together with inorganic phosphate. Although the molecular mechanisms of the MPT are largely unknown, the most widely accepted model (working hypothesis) is that it occurs after the opening of a channel complex that has been termed the permeability transition pore (PTP), which is thought to consist of the voltage-dependent anion channel (VDAC: outer membrane channel), the adenine nucleotide translocator (ANT: inner membrane channel), cyclophilin D (Cyp D), and possibly other molecule(s) [9] (see Fig. 2). However, it still remains uncertain whether the PTP really exists and what its exact nature is. Moreover, several experimental findings are difficult to explain by this model (see the introduction of [10]). A role of the ANT in the MPT is supported by MPT inhibition and activation by bonkrekic acid and atractyloside, respectively, which are ANT ligands [11]. Cyp D is a mitochondrial member of the cyclophilin family, which possesses peptidyl prolyl-*cis*, *trans*-isomerase (PPIase) activity and has a crucial role in protein folding [12]. The putative role of Cyp D in regulating the MPT is based on the observation that cyclosporin A (CsA), a specific inhibitor of the cyclophilin family, blocks the MPT [13]. Since CsA inhibits PPIase and the MPT at similar concentrations, PPIase activity may be critical for the MPT to occur. Cyp D resides in the mitochondrial matrix, but becomes associated with the inner mitochondrial membrane during the MPT. Based on the enzymatic activity of Cyp D as a PPIase, it may induce a conformational change of an inner membrane channel such as the ANT that leads to an increase of inner membrane permeability. In addition to the CsA-sensitive and Ca^{2+}-dependent ("regulated") MPT, the existence of a CsA-insensitive ("unregulated") MPT has also been suggested, although its mechanism and relationship to the CsA-sensitive MPT are totally unknown [10].

In the mid-1990s, the MPT attracted the attention of investigators in the cell death field, because it was reported that at least some forms of apoptosis could be inhibited by CsA, suggesting a role of the CsA-sensitive MPT in this process of cell death [9, 14]. A possible role of the MPT in apoptosis is also supported by the finding that apoptosis can sometimes be inhibited by bonkrekic acid [11, 15]. The CsA-sensitive MPT has also been implicated in remodeling of the mitochondrial cristae and mobilization of cytochrome *c* stores from the cristae during apoptosis, which promotes the complete release of cytochrome *c* [16]. However, the overall role of the MPT in apoptosis was still controversial, because there have been a number of reports that apoptosis

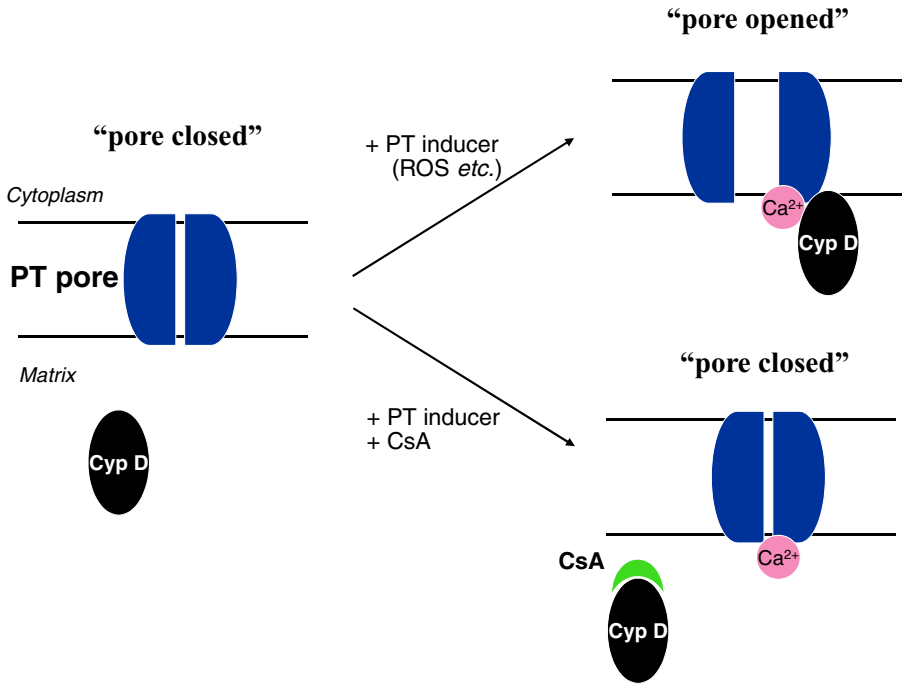

Fig. 2 Model of the MPT pore Under normal conditions, Cyp D is localized to the mitochondrial matrix, and the MPT pore is closed. In the presence of permeability transition inducers, Cyp D is considered to bind to and induce a conformational change of a channel in the inner membrane, resulting in opening of the MPT pore. Cyclosporin A (CsA) binds to and inhibits Cyp D to prevent MPT pore opening

is not inhibited by CsA [17]. Also, it has been demonstrated that $\Delta\psi$ occurs after cytochrome *c* release in at least some types of apoptosis, suggesting that the MPT is not always the trigger for cytochrome *c* release and cell death. This issue was recently been solved by studies performed in Cyp D-deficient mice, as discussed later.

Are the VDAC, the ANT, and Cyp D essential for the MPT?

It has long been thought that the VDAC, the ANT, and Cyp D play an essential role in the MPT, but convincing evidence was lacking until very recently.

An important role of the VDAC in the MPT has been supported by the following findings: (1) the electrophysiological properties of the PTP are strikingly similar to those of the VDAC incorporated in planar phospholipids bilayers [18, 19]; (2) various factors that alter VDAC channel properties, such as addition of NADH, Ca^{2+}, or glutamate, as well as binding to hexokinase II [20–24], also modulate PTP activity [25–27]; and (3) chromatography of mitochondrial extracts on a Cyp D affinity column leads to purification of the VDAC associated with the ANT [28].

The most convincing evidence about involvement of the VDAC in the MPT should theoretically be obtained by studies employing VDAC-deficient cells. Such a study was recently performed with mitochondria isolated from VDAC1-deficient cells, and it was found that VDAC1-deficient mitochondria still undergo the MPT normally, suggesting that VDAC1 is not important for this process. However, this result could have been due to compensation for VDAC1 deficiency by other isoforms, including VDAC2 and VDAC3. So far, experimental evidence for a direct role of the VDAC in the MPT has been provided by studies using specific anti-VDAC antibodies [29]. Two polyclonal anti-VDAC antibodies, which recognize different VDAC epitopes and inhibit its activity in liposomes [29], have been shown to inhibit the Ca^{2+}-induced MPT [29], supporting a crucial role for the VDAC in this process.

The ANTs (ANT1 and 2 in mice and ANT1, 2, and 3 in humans) are also considered to be important for the MPT. It has been demonstrated that Cyp D interacts directly with the ANT, although it is not known whether CsA inhibits this interaction [28, 30]. Regarding the role of the ANT in the MPT, considerable progress was made recently because it was shown that liver mitochondria from mice lacking both ANT1 and ANT2 still underwent the MPT, although the triggering Ca^{2+} concentration was slightly increased [31]. This finding suggests that ANT1/2 only play a limited role, if any, in the MPT or else that deficiency of ANT1/2 was compensated by other channel(s). The lack of an important role for the ANT in the MPT would be consistent with the observation that mitochondria isolated from yeast lacking the ANT still undergo MPT-like changes, including loss of membrane potential and swelling in response to ethanol, which are very similar events to those occurring in mammalian mitochondria during the MPT [32]. However, it is unknown whether

yeast mitochondria undergo a real MPT, because swelling of these mitochondria and loss of membrane potential in response to ethanol are not inhibited by CsA, although this inability of CsA to inhibit MPT-like events might be due to its inability to inhibit a Cyp D counterpart in yeast mitochondria. If the ANT is not involved in the MPT, the other channel(s) that are actually involved might be ANT-like inner membrane channels, because the MPT is modulated by ANT ligands such as bonkrekic acid or atractyloside and is accompanied by loss of $\Delta\psi$ (i.e., increased permeability of the inner mitochondrial membrane). Identification of one or more channels in the inner mitochondrial membrane that are directly involved in the MPT and might be targets of Cyp D would be an important step forward.

The role of Cyp D in the MPT was initially suggested by the finding that the MPT is blocked by CsA, which is known to inhibit the PPIase activity of cyclophilins. This finding has recently been confirmed by studies performed employing Cyp D gene (*ppif*)-deficient mice [33–36]. It has been demonstrated that Cyp D-deficient mitochondria isolated from the livers of these mice do not undergo the CsA-sensitive MPT in response to a variety of inducers, including Ca^{2+}, atractyloside, and H_2O_2. Because the MPT does not occur, these mitochondria accumulate a much higher concentration of Ca^{2+} than control mitochondria [33, 36]. However, the CsA-insensitive MPT (with loss of $\Delta\psi$ and swelling) can still occur when these Cyp D-deficient mitochondria are exposed to high concentrations of Ca^{2+} [33, 35]. In addition, Cyp D-deficient mitochondria show a normal response to reagents like ubiquinone and thiol oxidants that cause the CsA-insensitive MPT [35]. The CsA-sensitive MPT and CsA-insensitive MPT might share a common mechanism, because both forms of MPT are inhibited by ubiquinone 0 [35]. This finding might also suggest that Cyp D only sensitizes the mitochondria to the Ca^{2+}-induced MPT, although these two forms of MPT might be mediated by different mechanisms. This issue will only be solved by identification of the essential players involved in the MPT. In any case, it has been confirmed that Cyp D has a specific role in the CsA-sensitive MPT.

Although it is now clear that the Cyp D is an essential component of the CsA-sensitive MPT, there are still many questions to be answered. More studies are needed to elucidate the molecular nature of the MPT pore complex. Assuming that Cyp D interacts as a PPIase with other molecules essential for the MPT that probably reside in the inner mitochondrial membrane, a promising approach would be the isolation of a protein complex containing Cyp D and the VDAC. Another issue would be investigation of the relationship between the Cyp D-dependent MPT and the unregulated MPT. Furthermore, does the unregulated MPT have a role in apoptosis or other forms of cell death?

Role of the MPT

For a long time, it has been unclear whether the CsA-sensitive MPT plays an important role in the apoptotic increase of mitochondrial membrane permeability. However, studies of Cyp D-deficient mice have finally solved this issue. Various cells isolated from Cyp D-deficient mice, such as thymocytes, embryonic fibroblasts (MEFs), and hepatocytes, undergo apoptosis normally in response to various stimuli, including etoposide, stautosporine, and tumor necrosis factor-α [33–36]. Small intestinal cells from Cyp D-deficient mice are also as sensitive to X ray-induced apoptosis as cells from control mice [33]. These results provide the most compelling evidence that the CsA-sensitive MPT is not essential for apoptosis. Of course, it remains possible that some forms of apoptosis might be mediated by the CsA-sensitive MPT, and thus inhibited by CsA. However, the inhibitory effect of CsA on apoptosis might need to be more carefully evaluated because it is usually studied at relatively high CsA concentrations that could inhibit other targets, including cytoplasmic cyclophilins involved in transcriptional regulation, thus having a secondary effect on apoptosis. Accordingly, it may be necessary to re-evaluate CsA-dependent inhibition of apoptosis by using Cyp D-deficient cells or by silencing Cyp D to assess the real effect of CsA.

Several studies have indicated that overexpression of Cyp D protects cells against some forms of apoptosis. For example, the overexpression of CypD inhibits apoptosis induced by overexpression of caspase-8 (but not Bax) or by exposure to arsenic trioxide [37, 38]. It may be possible that these forms of apoptosis are mediated by the MPT, which is somehow affected by Cyp D overexpression. However, studies of transgenic mice with myocardial expression of Cyp D have revealed that cardiac myocytes isolated from these mice show a tendency to undergo mitochondrial swelling and spontaneous death [34], suggesting that the effects of Cyp D expression might be cell type-specific.

In contrast to the lack of any influence of Cyp D deficiency on apoptosis, the Cyp D-dependent MPT plays an important role in some forms of necrotic cell death (see Fig. 1). Cyp D-deficient MEFs show significantly increased resistance to H_2O_2-induced necrosis [33, 34], and Cyp D-deficient hepatocytes display resistance to necrosis induced by a Ca^{2+} ionophore (A23187) or by H_2O_2 [33, 34]. Interestingly, when H_2O_2-induced and Ca^{2+} ionophore-induced necrosis is inhibited by Cyp D deficiency in these cells, apoptosis does not occur as an alternate death mechanism [33], suggesting that the H_2O_2/Ca^{2+}-triggered apoptotic signaling pathways are somehow blocked in these types of cells.

Another very interesting question concerns the biological significance of the MPT because it is conceivable that the MPT plays a role in some physiological processes. By

analyzing Cyp D-deficient mice and cells in more detail, some hints about the role of the MPT should be obtained.

Regulation of the MPT by Bcl-2

Anti-apoptotic members of the Bcl-2 family, such as Bcl-2 itself and Bcl-x_L, are known to inhibit the Bax/Bak-dependent apoptotic increase of mitochondrial membrane permeability by direct interaction with pro-apoptotic members of this family, and also inhibit the MPT itself [39, 40] (see Fig. 1). How do these proteins block the MPT? Given that Bax/Bak is not essential for the MPT [33], Bcl-2 (Bcl-x_L) might directly inhibit a component of the PTP complex. In fact, Bcl-2 (Bcl-x_L) is capable of blocking VDAC activity [39] and ANT activity in liposome systems [41]. As described above, the VDAC plays a role in the MPT [29], whereas the ANT might not be important [31], so Bcl-2 and Bcl-x_L possibly inhibit the MPT by blocking the VDAC or unknown channels similar to the ANT that are actually involved in the MPT.

Role of the Cyp D-dependent MPT in disease

The advent of Cyp D-deficient mice has provided compelling evidence that the Cyp D-dependent MPT plays a crucial role in ischemia/reperfusion injury affecting the heart [33, 34] and brain [36], suggesting that the Cyp D-dependent MPT is involved in ischemia/reperfusion-induced cell death and that Cyp D and other components of the MPT are promising therapeutic targets. However, there have been a large number of reports published on the death mechanisms of ischemia/reperfusion injury and investigation of therapeutic methods, making it evident that ischemia/reperfusion injury is a very complex phenomenon which might involve multiple death mechanisms, because such injury can be suppressed by various inhibitors of different forms of cell death. It has been shown that ischemia/reperfusion injury can be ameliorated by inhibiting apoptosis with caspase inhibitors [42–45], inhibiting necroptosis with Nec1 [46], or blocking the Ask 1 pathway [47]. In studies of model systems employing cell lines, the death mechanisms involving caspases, a Nec1 target, Ask1, and the Cyp D-dependent MPT do not seem to overlap with each other. Why are so many different potential mechanisms involved in ischemia/reperfusion injury? Different death mechanisms might operate in the same cell in a sequential manner or in parallel, meaning that the inhibition of one mechanism might have a protective effect. Alternatively, different death mechanisms might act on different cells during ischemia/reperfusion injury and the dying cells might trigger the death process in other cells. It is also possible that different cell death mechanisms are activated by different ischemic conditions. For further studies of ischemia/reperfusion injury, mice that lack certain cell death mechanisms, such as Cyp D-deficient mice and Bax/Bak-deficient mice, would be useful tools.

The Cyp D-dependent MPT might also be involved in other diseases. It has been reported that mitochondria isolated from the livers of MND2 mice with mutation of the omi gene are more susceptible to the MPT [48]. MND2 mice succumb to motoneuron disease [49], which might be caused by the MPT occurring at a lower threshold in neuronal mitochondria. Thus, future studies may unveil a role of the Cyp D-dependent MPT in the pathogenesis of various diseases.

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