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Review

Is mPTP the gatekeeper for necrosis, apoptosis, or both?☆

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ABSTRACT

Permeabilization of the mitochondrial membranes is a crucial step in apoptosis and necrosis. This phenomenon allows the release of mitochondrial death factors, which trigger or facilitate different signaling cascades ultimately causing the execution of the cell. The mitochondrial permeability transition pore (mPTP) has long been known as one of the main regulators of mitochondria during cell death. mPTP opening can lead to matrix swelling, subsequent rupture of the outer membrane, and a nonspecific release of intermembrane space proteins into the cytosol. While mPTP was purportedly associated with early apoptosis, recent observations suggest that mitochondrial permeabilization mediated by mPTP is generally more closely linked to events of late apoptosis and necrosis. Mechanisms of mitochondrial membrane permeabilization during cell death, involving three different mitochondrial channels, have been postulated. These include the mPTP in the inner membrane, and the mitochondrial apoptosis-induced channel (MAC) and voltage-dependent anion-selective channel (VDAC) in the outer membrane. New developments on mPTP structure and function, and the involvement of mPTP, MAC, and VDAC in permeabilization of mitochondrial membranes during cell death are explored. This article is part of a Special Issue entitled Mitochondria: the deadly organelle.

1. Introduction

In recent years, the role of mitochondria in both apoptotic and necrotic cell death has received considerable attention. An increase in mitochondrial membrane permeability is one of the key events in apoptotic and necrotic death, although the details of the mechanisms involved remain to be elucidated. Unlike the resting potentials of neurons and cardiomyocytes, which are largely governed by basal K^+ conductance of their plasma membranes, the value of the mitochondrial resting potential is controlled by the very high resistance of the inner membrane, i.e., low permeability to all ions. While the inner membrane contains several channels, their opening is tightly regulated in order to prevent dissipation of the membrane potential and proton gradient that is the electrochemical energy reservoir for ATP-synthesis and transport. These channels include the putative K^+_{ATP} channel and mitochondrial Centum-picoSiemen (mCS), which are cation- and anion-selective channels that monitor metabolic levels and aid in volume regulation, respectively [1–3]. There are also inner membrane channels within the protein import complexes called TIM22 and TIM23 [4,5]. The inner membrane also expresses multiple calcium channel activities that range from the mitochondrial ryanodine receptor (mRyR) of cardiomyocytes to the more generic calcium uniporter [6,7]. Uncontrolled opening of any one of these channels may unleash havoc resulting in cell death. For example, exogenous targeting peptides putatively open the pore of the TIM23 complex and induce a rapid and high-amplitude swelling of mitochondria [8].

The mitochondrial permeability transition pore, or mPTP, is however the most notorious of all the inner membrane channels. mPTP has not only been linked to a rupture of mitochondrial outer membrane during cell death but also to a myriad of pathologies (Fig. 1) [9–11]. More recently, outer membrane channels such as the mitochondrial apoptosis-induced channel (MAC) and the voltage-dependent anion channel (VDAC) were also shown to be directly or indirectly involved in mitochondrial permeabilization during apoptosis and/or necrosis (Fig. 1) [12–15]. This review summarizes our current understanding of the role of the mPTP during mitochondrial membrane permeabilization and how this channel may collaborate with MAC and VDAC in order to regulate cell death.

2. Separating fact from fiction on the structure and function of mPTP

The mPTP was originally observed in swelling experiments on isolated mitochondria reported in landmark studies by Hunter and Haworth in 1979 [16–18]. The mPTP has a large caliper pore with low ion selectivity. Opening of the mPTP increases the permeability of the inner membrane for molecules up to 1.5 kDa that leads to organelle swelling and mitochondrial depolarization. The mPTP can

Abbreviations: CsA, cyclosporine A; ROS, reactive oxygen species; MPT, mitochondrial permeability transition; MOMP, mitochondrial outer membrane permeabilization; iMACs, inhibitors of MAC

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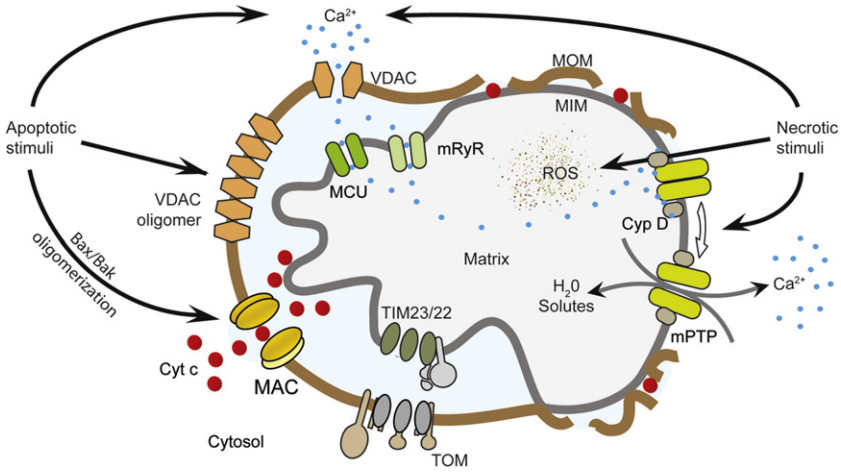

Fig. 1. Mitochondrial ion channels in apoptosis and necrosis. Left, apoptotic stimuli induce relocation of Bax from the cytosol into the mitochondrial outer membrane (MOM). Bax, Bak, and possibly other unidentified protein(s) oligomerize and form MAC to release cytochrome *c*. VDAC oligomerization upon apoptotic stimuli was also reported to be involved in cytochrome *c* release. Right, necrotic stimuli lead to exacerbated calcium uptake and reactive oxygen species generation by mitochondria. High levels of calcium and reactive oxygen species (ROS) induce a cyclophilin-D (Cyp D)-sensitive opening of mPTP that leads to swelling of the matrix and release of calcium. Swelling disrupts the outer membrane, while released calcium activates proteases, phosphatases, and nucleases that lead to necrotic degradation. Adapted from Ref. [112]. TIM23/22, translocases of the inner membrane mRyR, mitochondrial ryanodine receptor; TOM, translocase of the outer membrane; MCU, mitochondrial calcium uniporter; MIM, mitochondrial inner membrane.

be activated in cells and metabolizing isolated mitochondria by a myriad of effectors but, most notably, calcium plus phosphate and reactive oxygen species (see an extensive review in Ref. [19] and a more recent review in Ref. [20]). Reversible closure of mPTP occurs upon removal of calcium with EGTA or by the addition of ADP, magnesium, or cyclosporine A (CsA). The principal trigger for mPTP opening is matrix calcium in the presence of phosphate, but the activating concentrations of both are thought to rely on several other factors. For example, higher calcium levels are needed for mPTP opening in the presence of any one of several mPTP inhibitors including high negative membrane potential, low matrix pH, accumulation of adenine nucleotides like ADP, and other divalent cations like magnesium and strontium. Conversely, the calcium levels needed for mPTP activation are lower if adenine nucleotides are depleted or a mitochondrial uncoupler is added to depolarize the membrane potential after the uptake of calcium. Opening of the mPTP in response to oxidative stress has been linked to ischemia–reperfusion injury in the heart [9] and more recently to neurodegenerative diseases, like Alzheimer's and multiple sclerosis [10,11]. Hence, mPTP has become an important pharmacological target for both cardio- and neuroprotection.

The mPTP can also temporarily open or “flicker” leading to transient membrane potential variations in response to certain intracellular signals unrelated to cell death. Then, mPTP closure leads to a “resealing” of the inner membrane and a restoration of the ability to synthesize ATP [21]. This mode of action of the mPTP underlies a mitochondrial version of “calcium-induced calcium release” between the cytosol and the mitochondrial matrix [22–24]. That is, transient opening of mPTP could provide the pathway for mitochondrial Ca^{2+} extrusion under relatively normal conditions. The rapid removal of calcium from the cytosol and its subsequent release from mitochondria through mPTP flickering could prevent calcium inactivation of channels essential to refilling calcium stores as well as allow for signaling in these microdomains. Thus, mPTP flickering could impact processes as diverse as muscle contraction and saliva secretion [25,26].

Furthermore, brief opening of mPTP causes a transient mitochondrial depolarization and a short burst of ROS production and reveals a potential role for mPTP in ROS signaling [27]. ROS generated by one mitochondrion might then interact with neighboring mitochondria, perhaps once again transiently opening mPTP. The ensuing depolarization could produce additional ROS. Mitochondria-generated ROS has putative roles in a myriad of cell signaling as evidenced by the redox sensitivity of chaperones, kinases, and even gene expression [28]. Hence, mPTP can, at least indirectly, impact many cellular processes that are not linked to cell death. Nevertheless, flickering of the mPTP can result in sufficient production of ROS leading to sustained mPTP opening and eventually cell death [29].

Even though the phenomenon of mitochondrial permeability transition was originally described in the late 1970s, the molecular composition of the mPTP remains a mystery today. The biochemical studies of mitochondrial complexes by Brdiczka's group [30] and the electrophysiological studies of Zoratti's group [31] were among the first to propose that the complexes responsible for mPTP activity spanned both the inner and outer membranes of mitochondria. In fact, they were proposed to be in contact sites, or close junctions, between the two mitochondrial membranes.

These watershed studies also led to the hypothesis that the mPTP contains the outer membrane channel VDAC, which is also often referred to as mitochondrial porin [30,31]. More recent studies in which the three Vdac isoforms are knocked out have raised doubts about the essential nature of the involvement of outer membrane components in mPTP as these knockouts continue to express mPTP activity [32]. Nevertheless, VDAC continues to be purported as part of the mPTP mechanism in normal cells [33–35]. In other studies, VDAC oligomers were even proposed to be directly responsible for cytochrome *c* release (Fig. 1) [15,36].

The adenine nucleotide translocator, or ANT, has long been proposed to be an inner membrane component of mPTP. Some of these studies were biochemical, while others were pharmacological [30]. ANT inhibitors like bongkrekic acid and atractyloside lock the translocator in opposite conformations to prevent or induce a mitochondrial permeability transition, respectively [37]. ANT is the most abundant inner membrane protein on a molar basis and is frequently the target of misfolding. In this scenario, ANT undergoes large conformation changes and the atractyloside-stabilized conformation appears to be particularly vulnerable to damage leading to misfolding and mPTP formation (reviewed in Refs. [38,39]). However, the seminal studies of Wallace's group clearly showed the mPTP was in fact still present in Ant knockouts [40]. Hence, Ant does not provide the essential inner membrane permeability pathway for mPTP. Nevertheless, the adenine nucleotide translocator likely plays a regulatory role as the sensitivity of mPTP to certain pharmacological