REVIEW

The Pathophysiology of Mitochondrial Cell Death

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In the mitochondrial pathway of apoptosis, caspase activation is closely linked to mitochondrial outer membrane permeabilization (MOMP). Numerous pro-apoptotic signal-transducing molecules and pathological stimuli converge on mitochondria to induce MOMP. The local regulation and execution of MOMP involve proteins from the Bcl-2 family, mitochondrial lipids, proteins that regulate bioenergetic metabolite flux, and putative components of the permeability transition pore. MOMP is lethal because it results in the release of caspase-activating molecules and caspase-independent death effectors, metabolic failure in the mitochondria, or both. Drugs designed to suppress excessive MOMP may avoid pathological cell death, and the therapeutic induction of MOMP may restore apoptosis in cancer cells in which it is disabled. The general rules governing the pathophysiology of MOMP and controversial issues regarding its regulation are discussed.

The major form of apoptosis seen in most settings in vertebrate cells proceeds through the mitochondrial pathway, defined by a pivotal event in the process—mitochondrial outer membrane permeabilization (MOMP). MOMP occurs suddenly during apoptosis (*1*), leading to the release of proteins normally found in the space between the inner and outer mitochondrial membranes (including cytochrome c, AIF, and others). Before, during, or after MOMP, there is frequently a dissipation of the mitochondrial inner transmembrane potential ($\Delta\Psi m$), and the timing of MOMP versus $\Delta\Psi m$ loss can provide clues to the mechanism involved in a particular setting. MOMP precipitates the death of the cell through as many as three general mechanisms, including the release of molecules involved in the activation of caspases that orchestrate downstream events often associated with apoptosis, the release of molecules involved in caspase-independent cell death, and the loss of mitochondrial functions essential for cell survival (table S1). A pathophysiological role for MOMP is emerging.

Mechanisms of MOMP and the Decision to Die

The mechanisms responsible for MOMP during apoptosis remain controversial, although it is clear that many proteins can inhibit or prevent MOMP by local effects on mitochondrial membranes (tables S2 and S3). In general, two classes of mechanism have been described and each may function under different circumstances: those in which the inner mitochondrial membrane participates, and those involving only the outer membrane (Fig. 1).

In the first class of mechanism, a pore opens in the inner membrane, allowing water and molecules up to ~1.5 kD to pass through. Although most models of this pore, the permeability transition (PT) pore, postulate roles for the adenine nucleotide transporter (ANT) in the inner membrane and the voltage-dependent anion channel (VDAC) in the outer membrane (*2*), this is a hypothetical model (supporting online text). Recent evidence has shown that the PT pore can form in the absence of the ANT (*3*), and alternative models accounting for this pore have been proposed (*4*). Opening of the PT pore can be triggered by multiple stimuli and leads to (i) $\Delta\Psi m$ loss as ions equilibrate across this membrane, and (ii) swelling of the matrix as water enters. The latter can result in sufficient swelling to break the outer membrane to produce MOMP. It should be noted that although loss of $\Delta\Psi m$ accompanies irreversible PT, many other events can produce this loss. Loss of $\Delta\Psi m$ is not sufficient to prove the involvement of PT. Conversely, PT pore opening can be transient (through flickering of the pore), and therefore sustained $\Delta\Psi m$ does not provide a firm argument against the involvement of the PT pore unless monitored continuously throughout MOMP. In view of the difficulties of quantifying PT in living cells, and in the absence of a clear molecular substrate for the pore (supporting online text), it may be a pragmatic approach to define PT-associated MOMP as a process that can be inhibited by some ligands of putative PT pore constituents such as VDAC, ANT, or the ANT-interacting protein cyclophilin D (a target of cyclosporin A) (*5*). Ideally, methods that directly assess the permeability of the inner membrane (*6*) should be employed.

The second class of mechanism for MOMP does not involve a major role for PT or the mitochondrial inner membrane. This appears to be mediated by members of the Bcl-2 family of apoptosis-regulating proteins acting directly on the outer mitochondrial membrane (table S4). Anti-apoptotic Bcl-2 family members function to block MOMP, whereas the various pro-apoptotic members promote it. The latter fall into two general subfamilies, based on sharing of Bcl-2 homology (BH) domains. BH123 (or multidomain) proteins share BH1, BH2, and BH3 and appear to be effectors of MOMP, because cells from mice lacking the two major BH123 proteins, Bax and Bak, fail to undergo MOMP in response to a wide range of apoptotic signals (*7*). The other subfamily, the BH3-only proteins (that contain only the BH3 domains), can act either to activate Bax and Bak or to interfere with the anti-apoptotic Bcl-2 family members (*8*).

Vesicles composed of purified mitochondrial outer membranes can be permeabilized in response to activated forms of recombinant Bax or Bid, the latter presumably acting through Bak on the outer membrane (*9*). Further, vesicles composed of mitochondrial lipids (without other mitochondrial proteins) were permeabilized by recombinant, monomeric Bax, provided that active recombinant Bid or a BH3 peptide derived from Bid was present. This generated openings of indeterminate size that could not be visualized by conventional ultrastructural techniques. Such openings may be consistent with large lipidic pores composed of activated BH123 proteins and lipids with potential for negative curvature in membranes (*10*), for instance, the mitochondrial lipid cardiolipin. However, the presence of cardiolipin in the mitochondrial outer membrane remains controversial.

Some studies have implicated the outer membrane protein VDAC in MOMP. Bax and Bak can bind to VDAC, but possibly with different effects. Although the Bax-VDAC interaction is suggested to cause MOMP, interaction of Bak with VDAC-2 appears to be inhibitory (*11*). One possibility is that VDAC functions to sequester small amounts of cardiolipin or related lipids

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present in the outer membrane to microdomains in which local concentrations of these lipids may be sufficient to allow permeabilization of the membrane by activated Bax or Bak. This would also account for the apparent binding of Bax to VDAC.

PT-independent MOMP can be followed by secondary PT. In sympathetic neurons deprived of nerve growth factor, Bax-dependent, PT-independent MOMP associated with cytochrome c release causes caspase activation and apoptosis. However, in the presence of caspase inhibitors, such cells survive until $\Delta\Psi$m drops (*12*). Studies in which cyclosporin A blocks the $\Delta\Psi$m loss and commitment to death suggest that PT determines the point of no return in these cells (*13*).

Upon MOMP, proteins of the intermembrane space are released, although whether or not all proteins are released simultaneously remains controversial. One suggestion is that these proteins are differentially sequestered in the intermembrane space and that secondary events are required for the release of some of them (*14*, *15*). For example, remodeling of the matrix and inner mitochondrial membrane may be required for the release of cytochrome c in some cases (*15*), although in other cases this was not observed (*16*). Such remodeling has been suggested to be mediated by PT (*15*). Further, mitochondrial fission can occur around the time of MOMP (*17*) and proteins that regulate fusion or fission of mitochondria appear to affect which proteins can be released upon MOMP.

Irrespective of its mechanisms, MOMP can seal the point of no return of the lethal process by the release of caspase activators such as cytochrome c (table S1). Once activated, caspases can cause a rapid loss of mitochondrial functions. Upon MOMP, executioner caspases can cleave the NDUSF1 subunit of respiratory complex I, and mutation of its single cleavage site can preserve mitochondrial functions during apoptosis (*18*). This can delay plasma membrane events associated with caspase activation, including loss of plasma membrane integrity and externalization of phosphatidylserine, thus indicating an important role for disruption of mitochondrial function in apoptotic cell death. Nonetheless, even without caspase activation, MOMP generally results in cell death through the release of multiple caspase-independent death effectors, as well as loss of essential mitochondrial functions (table S1 and supporting online text).

Upstream of MOMP

Multiple distinct signaling pathways converge on MOMP (tables S2 and S3). Although some of the BH3-only proteins in the Bcl-2 family have the capacity to activate Bax and Bak or, conversely, inhibit the anti-apoptotic Bcl-2 family members, other molecules may have these properties as well. The tumor suppressor p53 acts, in part, to induce apoptosis by inducing expression of the BH3-only protein PUMA, and PUMA-deficient cells display a resistance to p53-mediated apoptosis (*19*, *20*). However, p53 can trigger MOMP and apoptosis in the absence of transcription, and this can occur through direct activation of Bax (*21*) or Bak (*22*) or through binding to Bcl-2 and Bcl-X_L, which blocks their activity (*21*, *23*). Resolving the role for this mechanism versus that of transcriptional regulation will be important in understanding the apoptotic function of p53.

An emerging theme is one of nuclear proteins functioning in the cytosol through direct interactions with Bcl-2 family proteins. Ku70, involved in DNA repair, can inhibit Bax (*24*). Another nuclear protein, TR3, binds Bcl-2 and perhaps promotes MOMP through this interaction (*25*). Histone 1.2, released from the nucleus upon X-ray–induced DNA damage, can trigger MOMP (*26*), perhaps through an interaction with Bcl-2 family members. Hexokinase can interact with VDAC, and this interaction may inhibit the ability of Bax to cause MOMP (*27*). Intriguingly, enforced expression of hexokinase together with the glucose transporter Glut-1 is sufficient to confer cell survival (*28*).

Alternatively, Bcl-2 family members may act independently of mitochondria and upstream of MOMP. Cells lacking Bax and Bak display reduced calcium efflux from the en-

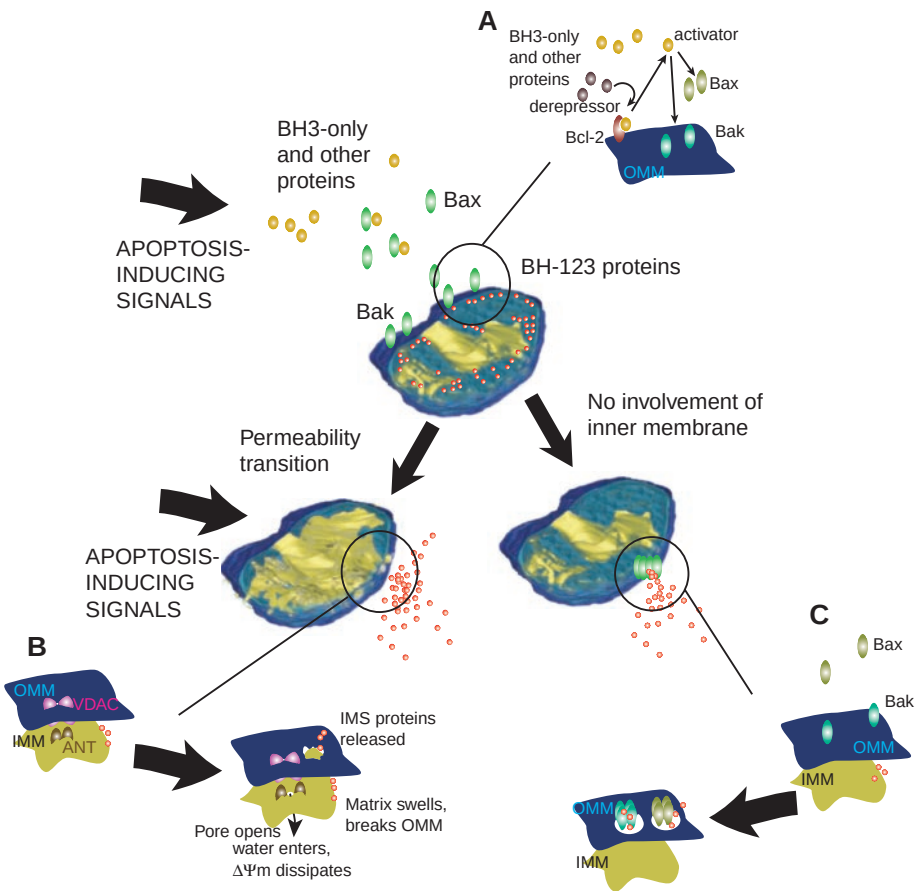

Fig. 1. Mechanisms for MOMP during apoptosis. (**A**) Signals for the induction of apoptosis (top) engage the activities of a subgroup of pro-apoptotic, BH3-only members of the Bcl-2 protein family and other proteins, which in turn activate the pro-apoptotic, BH123 proteins Bax and Bak to oligomerize and insert into the outer mitochondrial membrane (OMM). Other BH3-only proteins can act indirectly by releasing the first subgroup of BH123-activators from the anti-apoptotic Bcl-2 family proteins that sequester them. The BH123 proteins engage either of the two mechanisms that follow, perhaps depending on cell type or other conditions. (**B**) In PT-dependent MOMP, apoptosis-inducing signals act directly or indirectly to open the putative PT pore. This is composed of ANT or other proteins in the inner mitochondrial membrane (IMM) and is associated with VDAC and perhaps other proteins in the OMM. Opening the pore allows water to enter the matrix and ions to equilibrate, dissipating $\Delta\Psi$m at least transiently. The matrix swells, rupturing the OMM to release proteins of the mitochondrial intermembrane space (IMS). (**C**) In PT-independent MOMP, BH123 proteins, perhaps with other proteins, cause the formation of pores in the OMM through which IMS proteins are released. The mitochondrial tomograph was a gift from G. Perkins and D. Newmeyer. This was modified in the illustration.

Table 1. Examples of pathogenic processes involving excessive or deficient MOMP.

Disease	Pathogenic perturbation of MOMP	Pharmacological correction of deregulated MOMP
Ischemia reperfusion damage of brain or heart	Redox stress, excessive Ca^{2+} load, absent adenosine triphosphate and nicotine adenine denucleotide, and accumulating fatty acids favor PT and MOMP.	Bcl-2 inhibitors of the PT pore, as well as mito K^{ATP} channel openers, can exert neuro- or cardioprotective effects.
Neurodegenerative diseases	Respiratory dysfunction affects highly sensitive neurons in the central nervous system, leading to their premature death.	Putative inhibitors of the PT pore (minocyclin, rasagiline, and tauroursodeoxycholic acid) can prevent neurodegeneration.
Liver disease	Hepatotoxins (including bile acid and ethanol) and hepatitis B or C–encoded proteins induce MOMP.	Ursodeoxycholic acid prevents bile acid–induced PT and thus exerts hepatoprotective effects.
Cancer	MOMP-inhibitory proteins from the Bcl-2 family or unrelated proteins (such as Muc1) enhance apoptosis resistance.	Cytotoxic agents targeting Bcl-2–like proteins, PT pore components, and/or mitochondrial lipids enforce MOMP and kill cancer cells.
HIV-1 infection	Vpr, an accessory HIV-1–encoded protein, can act on ANT and Bax to trigger MOMP. This effect is frequently lost because of a mutation in long-term nonprogressors.	HIV-1 protease inhibitors can inhibit MOMP induced by Vpr in isolated mitochondria.

doplasmic reticulum in response to some stimuli (*29*) [whether this is directly an effect of Bax and Bak or an indirect effect of Bcl-2 on the receptor is controversial (*30*)]. The resulting calcium flux may act on mitochondria to produce MOMP independently of Bax and Bak, by induction of PT (*29*).

The Mitochondrial Pathway of Apoptosis in Pathogenesis

MOMP-dependent apoptosis is involved in major pathologies, with far-reaching medical and pharmaceutical implications (Table 1 and table S5). However, the role of MOMP in disease is often inferred from correlative studies, such as when a disease-associated molecule or pharmaceutical agent is shown to have effects on mitochondria, and should therefore be treated with caution. Nevertheless, the manifestations of MOMP (the mitochondrial release of intermembrane proteins, as well as the dissipation of $\Delta\Psi m$) are frequently observed in disease states with increased cell death (*31*).

Many viruses have acquired the capacity to intercept or to activate the principal signal-transducing pathways leading to cell death. Several proteins from pathogenic viruses are targeted to mitochondria and induce MOMP (Table 1 and table S5), and at least one is a virulence factor; a mutation in the human immunodeficiency virus (HIV) Vpr protein that attenuates its MOMP-inducing activity is statistically associated with a reduced risk to develop acquired immunodeficiency syndrome (AIDS) (*31*). Several oncogenic viruses encode MOMP-inhibitory proteins, and in humans these may be involved in the formation of virally induced lymphomas or Kaposi's sarcoma.

In acute pathologies, for instance after ischemia, a combination of increased intracellular Ca^{2+}, reactive oxygen species, and metabolic perturbations can trigger MOMP, which ultimately accounts for cell loss in cardiac infarction and cerebral stroke. This cell loss involves acute necrosis in the ischemic core and a slower apoptosis in the penumbra. The hypocampal CA1 region, extremely vulnerable to ischemia, contains mitochondria with the highest susceptibility to Ca^{2+}-induced MOMP in vitro (*32*). Key modulators of apoptosis, such as p53 and Bax, facilitate ischemia-induced MOMP and neuronal death (*33*), whereas the MOMP inhibitor Bcl-2 can prevent ischemia-induced neuronal apoptosis.

MOMP is also likely to be involved in chronic neurodegenerative diseases. For example, in Huntington's disease, polyglutamine expansions in huntingtin trigger neuronal death, and this aberration correlates with huntingtin-induced mitochondrial abnormalities. Transgenic overexpression of Bcl-2 can prolong the life span of mice carrying an SOD-1 mutation found in patients with familial amyotrophic lateral sclerosis, a degenerative disease affecting spinal motor neurons. Stabilization of mitochondrial membranes by genetic or pharmacologic manipulations also suggests a role for MOMP in neurodegeneration (table S5).

MOMP is also involved in acute toxin-induced cell death. Toxins implicated in Reye's syndrome, including salicylate, adipic, isovaleric, 3-mercaptoproprionic, 4-penenoic, and valproic acids, cause MOMP when added to purified mitochondria or to hepatocytes. Other prominent examples of toxic MOMP inducers include ethanol, CCl_4, and heavy metals or their organic derivatives (table S5).

MOMP induction is a therapeutic goal in cancer therapy. MOMP is regulated by several oncogene products, in particular the anti-apoptotic Bcl-2 family proteins, whereas several tumor suppressors induce or favor MOMP. A relative resistance to MOMP is a prominent hallmark of cancer (*34*). Experimental drugs that act on mitochondrial proteins or lipids have been shown to be therapeutic in preclinical mouse models (table S5), and some therapeutic treatments have been reported to induce MOMP, although it is not clear if direct MOMP induction accounts for their anticancer effects (table S6).

There is a great interest in developing drugs that prevent MOMP and suppress pathological cell death (table S5). Under some circumstances, MOMP is delayed or inhibited by cyclosporin A (CsA), and this can reduce the lethal effects of heavy metals or high-dose paracetamol in animal models (*35*). CsA or Bcl-2 can reduce infarct size in the heart and brain (*2*). CsA can also be used to enhance the functional recovery after hypothermic heart preservation (*36*). Several neuroprotective drugs also prevent Bax-mediated MOMP in isolated mitochondria: tauroursodeoxycholic acid probably through inhibition of Bax insertion (*37*) and dibucaine and propranolol at a later step that may involve outer membrane lipids (*38*).

Concluding Remarks

One particularly intriguing aspect that emerges from the complexity of MOMP regulation is the functional and/or physical interaction between apoptosis regulators (e.g., the Bcl-2 family) and proteins known to participate in intermediate metabolism, e.g., VDAC, hexokinase, or glucokinase. For example, the latter interacts with the pro-apoptotic protein Bad (*39*). These interactions may tie specific metabolic demands to apoptotic control and thus determine "metabolic windows" for cells to

avoid MOMP. In the absence of growth factors that regulate metabolite flow (*40*) or in conditions distant from optimal metabolic conditions (oxygen tension, redox potential, tissue pH, and glycolytic substrates), cells may be primed for MOMP and demise. This crosstalk between apoptosis and metabolism may contribute to the metabolic signature of cancer, the Warburg phenomenon, an increased reliance on anaerobic metabolism even in the presence of abundant oxygen. Progress at the frontiers of pathobiology will help to integrate the process of MOMP and its regulation into the physiology of the cell.

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41. Supported by Agence Nationale pour le Recherche sur le SIDA, European Commission, Ligue Nationale contre le Cancer, and the French Ministry of Science (G.K.) and by NIH and Gemini Science (D.R.G.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/305/5684/626/DC1
SOM Text
Tables S1 to S6
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Science **305**, 626 (2004);
DOI: 10.1126/science.1099320