Review

Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario

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Abstract

Mitochondrial outer membrane permeabilization (MOMP) is considered the 'point of no return' as this event is responsible for engaging the apoptotic cascade in numerous cell death pathways. MOMP is directly governed by a subset of the BCL-2 family of proapoptotic proteins, which induce disruptions in the outer mitochondrial membrane (OMM) and subsequent release of death-promoting proteins like cytochrome *c*. The proposal here is centered on our hypothesis that MOMP is dictated by an interaction between the cytosol and the OMM, and although proteins of the OMM may be important in the process, the 'decision' to undergo apoptosis originates within the cytosol with no participation (in terms of yes, no and when) by mitochondria.
Cell Death and Differentiation (2006) **13,** 1396–1402.
doi:10.1038/sj.cdd.4401963; published online 19 May 2006

Apoptosis and Caspase-Independent Cell Death: Where MOMP Happens

Apoptotic stimulation occurs through distinct signaling cascades.[1,2] The intrinsic (or mitochondrial) pathway integrates signals generated by a variety of stressors, including DNA damage, cytoskeletal damage, endoplasmic reticulum stress, loss of adhesion, growth factor withdrawal, macro-molecular synthesis inhibition and others. The characteristics of apoptosis (e.g., DNA laddering and chromatin condensation, loss of plasma membrane asymmetry and blebbing) are dependent on the activation of cysteine proteases (the caspases) that cleave numerous specific cellular substrates. The activation of caspases in the mitochondrial pathway requires mitochondrial outer membrane permeabilization (MOMP), an event that is considered to be the 'point of no return' during apoptosis as it results in the diffusion to the cytosol of numerous proteins that normally reside in the space between the outer (OMM) and inner (IMM) mitochondrial membranes (Figure 1a).[3] Among these is cytochrome *c*, which serves as a cofactor for apoptotic protease-activating factor-1 (APAF-1) to trigger the formation of the apoptosome and subsequent activation of the initiator and executioner caspases, normally caspase-9 and -3, respectively.

In order for a mitochondrion to undergo MOMP, a coordinated effort between numerous BCL-2 proteins (discussed below) must be engaged to allow for permeabilization of the OMM (Figure 1b). This permeabilization is likely achieved by the formation of membrane-spanning pores through which the intermembrane space proteins are released. Once MOMP has occurred, the cytosolic machinery responds by activating caspases, or if this pathway is inhibited, a caspase-independent cell death (CICD) process ensures cellular demise. This process may utilize less tractable mechanisms, such as ROS (reactive oxygen species), loss of mitochondrial function or released mitochondrial intermembrane space proteins such as apoptosis-inducing factor or endonuclease G to catalyze death.[4] In both situations, caspase-dependent and -independent cell death, MOMP occurs. This disrupts mitochondrial function and, even in the absence of caspase activation, energy production eventually wanes and the cell is left to die.[5]

The BCL-2 Family of Proteins: Sensors for Life and Death

Members of the BCL-2 protein family are distinguished by the presence of up to four different BCL-2 homology domains (designated BH1–4).[6] Generally speaking, those that contain all four domains (BCL-2, BCL-xL and MCL-1) are antiapoptotic, whereas those that contain less are proapoptotic (Figure 2). The proapoptotic group can be further divided into two groups, multidomains or BH123 members such as BAX and BAK, and BH3-only proteins, including BID, BIM and PUMA, among others. The multidomain proapoptotic BCL-2 proteins, BAX and BAK, are constitutively expressed and only induce MOMP following apoptotic stimuli, suggesting that they are inactive in nonapoptotic cells.[7,8] Activation by certain BH3-only proteins is required for BAX or BAK to
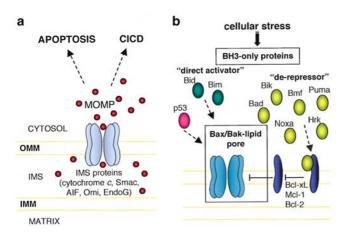
Figure 1 (a) Schematic representation of MOMP. Diverse forms of cellular stress activate the mitochondrial pathway by inducing the formation of pores in the OMM. Permeabilization of the OMM allows for the release of numerous intramembrane space proteins, which results in the activation of caspases and apoptosis; or it may induce death in a caspase-independent manner (CICD). (b) The BAX/BAK-lipid pore model. Direct activator BH3-only proteins or p53 induce BAX or BAK to form pores in the OMM. BCL-2, BCL-xL and MCL-1 can block this process at the OMM and are, in turn, regulated by de-repressor BH3-only proteins
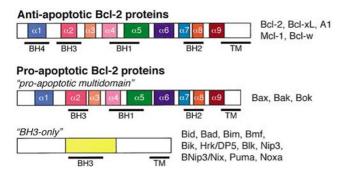

Figure 2 The BCL-2 family of proteins. The BCL-2 family of proteins is divided into antiapoptotic and proapoptotic members. The antiapoptotic members include BCL-2, BCL-xL, A1, MCL-1 and BCL-w and contain four BCL-2 homology domains (designated BH1–4). The proapoptotic multidomains (BAX, BAK and BOK) contain BH1–3 domains. The BH3-only proteins are structurally diverse and contain only one conserved domain, the BH3. The alpha helices of each protein are designated and the regions contained within each BH domain are illustrated by bold lines under each protein. The hydrophobic carboxyl-terminal transmembrane domain (TM) of each protein is based on in silico predictions and/or structural data and is not necessarily present in each member

Figure 3 Two mechanisms of proapoptotic multidomain activation by BH3-only proteins. (a) Central to MOMP is the activation and oligomerization of BAX or BAK. These proteins, once activated by a BH3-only protein, are what create pores in the OMM that permit the release of intramembrane proteins to the cytosol. (b) Direct activator BH3-only proteins, for example, BIM and BID, can induce the oligomerization and activation of BAX or BAK in the absence of other proteins. Through a transient interaction with BAX or BAK, the direct activator BH3-only proteins (BID is shown in this example), or peptides derived from the BH3 region, induce MOMP and cytochrome c release. (c) A subset of BH3-only proteins, the de-repressors, cannot induce the activation of BAX or BAK alone. In this scenario, a direct activator BH3-only protein is sequestered by an antiapoptotic BCL-2 protein. Following stress, a de-repressor BH3-only protein is induced, either by transcriptional upregulation or post-translational modification, and this protein then binds to an antiapoptotic BCL-2 protein promoting the release of a sequestered, direct activator BH3-only protein. In this example, BIM is sequestered by BCL-xL and the induction of BAD allows for the release of BIM to engage MOMP

oligomerize and insert stably into the OMM, an important prerequisite for MOMP (unlike cytosolic BAX, BAK is constitutively mitochondrial; but like BAX, inserts into the membrane only upon activation). Korsmeyer and colleagues demonstrated that cells from mice lacking both *bax* and *bak* are resistant to a wide range of proapoptotic insults underscoring the importance of these proteins in the mitochondrial pathway.[9] This is also highlighted by recent studies in growth factor-deprived *bax/bak* double-knockout cells where survival is maintained by marked autophagy owing to the absence of MOMP.[10] This suggests that when permeabilization is inhibited, cells respond by maintaining a minimal level of survival to be rescu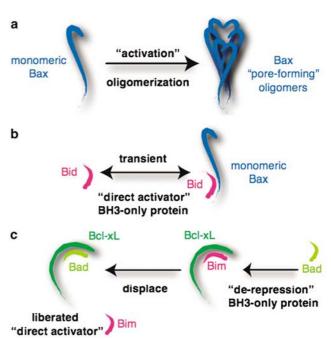ed in the event of growth factor or nutrient re-addition. Thus, in the absence of multidomains, and therefore MOMP, the default cellular program is to sustain viability.

In order to induce death, proapoptotic multidomain proteins must be activated by the BH3-only proteins (Figure 3a). A hypothesis has recently emerged that members of the BH3-only family of proteins can be divided into two distinct groups: 'direct activators' that directly activate BAX or BAK (Figure 3b), and 'de-repressors' (or 'sensitizers') that allow BAX or BAK to be activated by sequestering the antiapoptotic proteins such as BCL-xL or MCL-1 and allowing for the subsequent release of previously inhibited direct activators (Figure 3c).[7,11] Evidence suggests that the BH3-only proteins, BID and BIM, act by associating with BAX and BAK, but this seems to be transient as it is not detectable in the absence of detergent. Yet, the antiapoptotic proteins, BCL-2, MCL-1 and BCL-xL, inhibit this interaction by binding to BID and BIM. The other members of the BH3-only family (e.g., Noxa, BMF, HRK, BAD and BIK) are unable to activate directly BAX and BAK, but can bind to BCL-xL, BCL-2 and/or MCL-1 to various degrees. The direct activator/de-repressor model suggests

that the latter proteins release BID and BIM from their antiapoptotic partners enabling activation of BAX and BAK in an indirect manner. However, another interpretation of these results is that the BH3-only proteins function to antagonize antiapoptotic BCL-2 family members, rather than engage BAX or BAK.[12] This notion explains why combinations of the BH3-only proteins are required to induce cell death as each demonstrates selectivity in binding prosurvival targets. It is important to note that the majority of BH3-only protein literature is based on data derived by using only the BH3 peptide of the corresponding protein and not on a full-length, biologically relevant protein. Similar to a BH3-only protein, p53 can function to activate directly BAX, while it also has been described to bind to BCL-xL and BCL-2 similar to a de-repressor BH3-only protein.[13–15] Importantly, as only three direct-activator proteins have been described (BID, BIM and p53), other proteins may also have this function(s).

Thus, there seems to be a series of checks and balances in the cytosol where activation of proapoptotic multidomain proteins not only requires a direct activator BH3-only protein but also repression of the antiapoptotic BCL-2 family members by additional BH3-only proteins (perhaps also through transcriptional downregulation or enhanced protein degradation). So, what constitutes the commitment step that, once taken, will result in MOMP? Is it the activation of BAX/BAK or the induction of the BH3-only protein function? The BH3-only proteins become activated in response to different stimuli specific for each member of the family, and hence their regulators serve as primary 'sensors' for cellular stress. BID is activated upon cleavage by caspase-8, granzyme B and more weakly by caspase-2 and -3 and is engaged in response to death receptor stimulation, cytotoxic T-lymphocyte killing and heat shock, respectively.[16–21] BIM, on the other hand, is held inactive in the cell through binding to the dynein light chain-1 (DLC1) and may only activate multidomains upon release from the cytoskeleton.[22] Other BH3-only proteins such as BAD are activated by dephosphorylation, whereas PUMA and Noxa are transcriptionally upregulated by p53 and other proapoptotic stimuli.[23,24] In the presence of antiapoptotic BCL-2 proteins, activation of a direct activator BH3-only protein is generally not considered sufficient to induce MOMP as it will be sequestered by antiapoptotic proteins, and hence one or more of the de-repressor BH3-only proteins is also required. It is therefore likely that the same stimuli that activate BID or BIM also activate collateral de-repressor BH3-only proteins. For example, BMF also binds to myosin motor complexes (DLC2) and thus may detect similar cytoskeletal changes as BIM.[25] However, activation of BH3-only proteins is probably not sufficient to ensure that every cell will initiate MOMP. This is certainly the case in many human cancers where BCL-2 is overexpressed conferring resistance to chemotherapy.[26] It is likely that multiple signaling cascades are required for initiating MOMP, perhaps through the combined efforts of transcriptional regulation and complex post-translational modification (cleavage, phosphorylation, etc.). Considering the complexity and irreversibility of MOMP, we suggest that unrestrained activation of BAX or BAK is the ultimate commitment to cell death.

MOMP: (How) Are Mitochondria Really Involved?

By definition, MOMP occurs in the OMM, but this does not afford us with an explanation of how it occurs. There are various ways in which mitochondria may regulate their own permeabilization and these originate from either the IMM or OMM.

The inner membrane

The inner membrane may either cause or control MOMP through involvement of the mitochondrial permeability transition (mPT) pore.[27] The mPT pore is a complex composed of several different proteins including VDAC (voltage-dependent anion channel), ANT and cyclophilin D (cypD), which span the IMMs and OMMs where ANT is on the inner membrane and VDAC is on the outer membrane (Figure 4a). Opening of this pore allows for an influx of ions and other small molecules into the mitochondrial matrix causing swelling of the matrix, inducing rupture of the OMM and thus MOMP. The mPT pore has been implicated as being responsible for MOMP in certain scenarios including conditions of ER stress or ROS. It has been proposed that during ER stress-induced apoptosis, Ca^{2+} is released from the ER and is taken up by mitochondria resulting in cytochrome c release and apoptosis. Ca^{2+}-induced cytochrome c release from mitochondria appeared to occur in the absence of BAX and BAK (although BAX and BAK may function to control Ca^{2+} release from ER) suggesting mPT pore involvement.[28] However, under physiological conditions, the amount of calcium released from the ER is not sufficient to induce mPT in mitochondria, leading to the assumption that Ca^{2+}-induced mPT occurs in those mitochondria that are proximal to the ER. If so, then MOMP should only occur in these settings in a small subset of mitochondria,
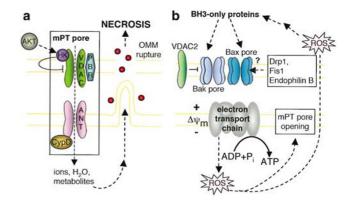

Figure 4 (**a**) Schematic representation of the mPT pore model. The hypothetical mPT pore is comprised of VDAC, ANT and a number of other proteins. Opening of the pore allows for an influx of water and ions into the matrix inducing swelling and rupture of the OMM and most likely results in necrosis. Various proteins have been suggested to regulate the mPT including hexokinase (HK) and the PBR. (**b**) Other proteins have been suggested to regulate MOMP, specifically the formation of the BAX (or BAK) pore including VDAC2 and proteins that regulate mitochondrial fission and fusion. Further, mitochondrial components may independently induce MOMP including ROS produced by the electron transport chain, which may cause opening the mPT pore, but equally may induce apoptosis via activation of BH3-only proteins in the cytoplasm

a prediction that is not supported by direct observations of cytochrome c release[29,30] (unpublished observations).

Recently, a number of genetic models have called into question the importance of mPT as a general inducer of MOMP. ANT seems to be dispensable as mice lacking ANT can still undergo mPT, whereas cells that lack cypD (therefore have no mPT) showed no difference in Ca^{2+}-induced or a variety of other forms of apoptosis, although Ca^{2+}-induced necrosis was defective.[31–33] These results from the *Ppif* KO mice (the gene encoding cypD) suggest that mPT may not be required during apoptosis (in most cases at least) and does occur during ischemia and ROS-induced necrosis; however, the role and mechanism of death remains unknown.[31] Furthermore, as mPT is highly temperature sensitive, the coordinated release of cytochrome c at different temperatures argues against any sort of mPT chain reaction.[5,29] Taken together, these data suggest that the mPT pore, although important for necrotic death, may not be necessary for MOMP that occurs during mitochondrial apoptosis (Figure 4a).

The inner membrane may alternatively control MOMP by regulating OXPHOS (oxidative phosphorylation), which in turn regulates the mitochondrial transmembrane potential $(\Delta\psi_m)$.[34] The maintenance of $\Delta\psi_m$ is required for a variety of mitochondrial functions including protein import, ATP production and regulation of metabolite transport. The onset of MOMP is often associated with a loss of $\Delta\psi_m$, but does this loss of $\Delta\psi_m$ induce MOMP or does it occur as a result of MOMP? Disruption of $\Delta\psi_m$ caused by incomplete reduction of molecular oxygen during OXPHOS leads to ROS generation that triggers MOMP, but this may occur via an interaction of ROS with as yet unidentified cytosolic sensors. Indeed, ROS that is generated during apoptosis is often caused by caspase-dependent cleavage of a subunit of complex I of the respiratory chain and hence can be a byproduct of MOMP rather than causative.[34,35] Otherwise, there is no compelling evidence that loss of $\Delta\psi_m$ directly induces MOMP, and OXPHOS can clearly continue after MOMP since, in the absence of caspase activation, not only can $\Delta\psi_m$ be regenerated, but the cells also can maintain ATP production.[34–36]

The outer membrane

Compelling evidence that the inner membrane (and indeed the matrix) is not necessary for MOMP and that MOMP only requires the OMM has been provided by Kuwana et al.[8] Using a reductionist approach to dissect the requirements for MOMP, they demonstrated that unilamellar vesicles comprised of the lipids present in the mitochondrial membrane release their contents in the presence of activated BAX. The use of defined liposomes and recombinant BCL-2 family proteins here may represent 'simplified' mitochondria, suggesting that the only mitochondrial requirement for the release of intermembrane space proteins is cardiolipin, a lipid that is specific to mitochondrial membranes. As no other proteins are required to release the liposome contents, these observations favor a mechanism of MOMP where activated BAX (and/or BAK) forms a pore in the lipid membrane to allow release of cytochrome c. However, this simplified liposome system may not accurately reflect the complexity of the permeabilization

process as it occurs in the native OMM and certain stimuli may require OMM proteins to allow for the formation of the BAX/lipid pore. In the absence of detergent, BAX requires membranes (cardiolipin-containing liposomes or mitochondria) to oligomerize and activate, and evidence suggests that BCL-xL will only inhibit BAX in the presence of similar lipid membranes.[8] However, the importance of cardiolipin in this process is also controversial. In yeast, cardiolipin does not seem to be required for BAX-induced death.[37] Moreover, the presence or exact amount of cardiolipin in the OMM is not known and it may be present in, at best, very low levels. Immunogold staining experiments suggest that cardiolipin in the outer membrane is concentrated at the contact points between the IMM and OMM where BAX/BID are proposed to bind, and perhaps the local concentration of cardiolipin at these sites may be sufficiently high to allow binding of these proteins.[38,39] Alternatively, there may be OMM proteins that concentrate cardiolipin, allowing for the insertion of activated BAX and/or BAK into the OMM to mediate MOMP.

There are two further levels of potential regulation that exist in the physiological context of an intact OMM (Figure 4b). First, proteins associated with the OMM, in addition to BCL-2 family members may directly regulate MOMP; and second, perhaps additional proteins associated with the OMM, also in addition to BCL-2 family members, participate in MOMP but do not regulate the process. For example, many of the proteins that have been described to regulate the mPT pore also have claims to participate additionally in MOMP. This is where a subtle difference, and perhaps, overlooked aspect of MOMP arises. Although there are numerous proteins in the OMM, most of these are not required for BAX or BAK to permeabilize mitochondria. Outer membrane vesicles prepared from mitochondria permeabilize upon treatment with tBID similar to intact mitochondria; this must be independent of mPT pore function as these are devoid of mitochondrial inner membrane constituents.[8] Yet, several proteins at the OMM are speculated to regulate BAX or BAK-mediated permeabilization because of a demonstrated association with the latter proteins. For example, BCL-2, BCL-xL, BAK and BAX have all been shown to bind VDAC, although it should be noted that as VDAC is the most abundant protein of the OMM, the binding may not be physiological.[40–43] More specifically, Korsmeyer and colleagues suggested that VDAC2 inhibits BAK activation by maintaining BAK as a monomer in the OMM (Figure 4b).[44] While this may be the case, VDAC2 may not make the decision to activate or inactivate BAK since in this scenario it is a cytosolic signal response to cellular stress, most likely a BH3-only protein, that disrupts the VDAC2–BAK association.[44]

Similarly, it has been suggested that the peripheral benzodiazepine receptor (PBR) blocks MOMP.[45,46] This integral membrane protein functionally interacts with the mPT pore and is a suggested inhibitor of MOMP. Yet, the requirements for cell death still do not change, both cellular stress and BH3-only proteins are necessary, as simple pharmacological inhibition or disruption of PBR activity is not sufficient for the mitochondria to broadcast a proapoptotic signal; and use of inhibitors certainly may not reflect the physiological functions of these proteins.[46] Such proteins (e.g., VDAC1/2 and PBR) may associate with numerous

BCL-2 proteins, but the signal for MOMP cannot originate from within mitochondria; the cell must generate a proapoptotic message that feeds forward (stress→cellular detection→cellular response→activation of BH3-only proteins→ BAX/BAK activation→MOMP) to the mitochondrial surface to regulate these interactions.

A description of such a 'feedforward' situation considers the role of AKT and hexokinase I/II in the prevention of cytochrome *c* release in certain survival pathways. AKT activity seems to be required to balance the requirements for glucose uptake, and subsequent metabolite transport across both the IMM and OMM by directly regulating the expression and localization of hexokinase on the OMM.[47,48] Interestingly, AKT activity increased the mass of hexokinase on the surface of mitochondria, which has been demonstrated to influence dramatically VDAC channel activity.[47] When cells were treated with agents that disrupted the hexokinase–mitochondria association, accelerated cytochrome *c* release occurred only following additional proapoptotic stress.[47] Furthermore, a reduction of BAX translocation has been reported during instances of enforced hexokinase–mitochondrial association during stress.[47] Together, this indicates that simple removal of hexokinase from the OMM is not sufficient to induce MOMP as the cells still do not release cytochrome *c* in the absence of stress, and that the signal to recruit BAX to the OMM must originate outside mitochondria regardless of hexokinase participation.

Another major class of proteins that reside in the OMM is responsible for mitochondrial dynamics: the fusion and fission proteins (Figure 4b). Mitochondrial dynamics are required for distributing mitochondria to daughter cells following mitosis and ensure that mitochondrial integrity is preserved as mitochondrial membranes divide and fuse.[49] There are at least four OMM proteins that potentially participate in, or regulate MOMP: DRP-1 (a dynamin-related GTPase), endophilin B1 (a lipid transferase required for determining membrane curvature), Fis-1 (and integral OMM protein) and Fzo1/Mfn1 (a large transmembrane GTPase).[50]

Thus far, it has been described that BAX and BAK, upon activation, coalesce with mitochondrial scission foci that are comprised minimally of DRP-1 and mitofusin-2, and that BAX can physically interact with endophilin B1 in the OMM; yet, dominant-negative forms of DRP-1, such as GTPase-deficient DRP-1[K38A], do not block the translocation of BAX following activation by a BH3-only protein.[51–53] Again, the signal responsible for promoting BAX translocation to the OMM is generated as a consequence of the particular cellular stress (i.e., the activated set of BH3-only proteins). But, what is the purpose of BAX/BAK interacting with this class of proteins? Do these proteins simply participate as 'docking points' for BAX or BAK, or is there an active regulation of MOMP involved? Upon activation, BAX must be able to target the appropriate intracellular membrane (i.e., the OMM) in order to engage the apoptotic cascade and these proteins may serve to 'dock' BAX to the OMM. If true, this interpretation could not be extended to include BAK as it is constitutively resides in the OMM. Yet, perhaps regulators of mitochondrial dynamics (such as DRP-1 or Fis-1) have evolved to participate in MOMP by directing the action of BAX and BAK to the appropriate region of the OMM allowing for

their pore-forming activity. In this scenario, the proposed interaction does not itself serve to make the cellular decision to induce MOMP, but it is still required. A reciprocal interaction has also been reported where antiapoptotic BCL-2 members promoted the remodeling of the mitochondrial network via a mitofusin-2 interaction that resulted in mitochondrial fusion and decreased sensitivity to cell death.[54] In this scenario, it was shown that CED-9, the *Caenorhabditis elegans* BCL-2 relative, could induce mitochondrial clustering. Similar mitochondrial reorganization was also induced by enforced BCL-xL expression, suggesting that this activity may be a conserved function of the BCL-2 family.[54]

Another potential aspect to this scenario relates to the specific lipid requirement for BAX (and presumably, BAK) to permeabilize an OMM. As discussed earlier, *in vitro* data suggest that BAX requires cardiolipin to oligomerize and engage pore-forming activity.[8] As cardiolipin primarily localizes in the IMM, does the fusion/fission machinery also regulate BAX-mediated MOMP by creating the appropriate lipid milieu at the contact sites with DRP-1 or Fis-1? Endophilin B1, a lipid transferase, interacts with BAX and may serve to re-distribute IMM lipids to contact sites for efficient activation of BAX (and by extension, BAK).[55,56] If this activity is required, then endophilin B1 may serve as a *bona fide* regulator of MOMP as its necessary function may itself be subject to mitochondrial dynamics and energetics. The issue is whether or not regulation of endophilin B1 ever helps to determine if and when MOMP will occur.

The Innocent Bystander Scenario

Given the evidence outlined here that the most likely mechanism for MOMP is the BAX/BAK–lipid pore with no further requirement for proteins in the IMM or OMM, we propose this possibility: mitochondria are essentially 'innocent bystanders' in the process of MOMP. Thus, it is a process entirely controlled by the BCL-2 family of proteins and their regulators within the cytosol. Although it is possible that proteins associated with mitochondria directly and indirectly regulate the BCL-2 family to induce MOMP, we suggest that it is still the activation of BAX and BAK that exclusively engages MOMP, and the control of this activation is what produces apoptosis. No other events other than opening the OMM regulate this step in apoptosis.

This is important to note because following the discovery of MOMP, a logical error occurred, as follows. Permeabilization of the OMM causes apoptosis and subsequently 'damages' mitochondria by physical disruption and eventual waning of mitochondrial function. Ergo, it is assumed that mitochondrial damage, in any form, causes apoptosis. The innocent bystander scenario suggests that MOMP is a very specific sort of mitochondrial damage, caused exclusively by BAX and BAK. Other forms of mitochondrial damage (perhaps to the outer or inner membranes, or targeted to mitochondrial DNA, etc.) will not signal apoptosis simply by virtue of the damage, but may signal other forms of cell death such as necrosis. If apoptosis does occur subsequent to these forms of damage, it is through other signaling events, such as ROS generation, that are sensed by the cytosol or nucleus to generate

additional signals that directly activate BAX and BAK. From this point of view, mitochondrial damage is no different from other forms of damage within a cell (e.g., genotoxic stress), and does not represent an alternative to BAX/BAK-controlled MOMP. An elegant study that examined the release of cytochrome c following partial mitochondrial damage supports this model.[57] In this system, mitochondria were labeled with a photosensitizer (Chloromethyl-X-Rosamine) and then a small subset of mitochondria was photoirradiated. Within the cell, the entire mitochondrial population released cytochrome c in a rapid and coordinated manner, suggesting that a signal (probably ROS) between the irradiated and nonirradiated mitochondria was generated; this signaling also occurred in the absence of waves (one mitochondrion communicating with its neighbor), suggesting a cytosolic sensor leading to coordinated BAX/BAK activation throughout the cell (a BH3-only proteins) was emanated to the entire mitochondrial population.

The innocent bystander hypothesis discussed here should not be confused with an alternative viewpoint that mitochondria simply serve to amplify the apoptotic signal downstream of caspase activation.[58] Despite recent evidence from the caspase-3 and -7 double-knockout mice, which suggests that caspases may act to induce BAX translocation and cytochrome c release, there is a sustained and clear literature that supports the requirement for cytochrome c and APAF-1 in the activation of caspases.[59,60] Furthermore, numerous rigorous studies have demonstrated that cytochrome c release from mitochondria is complete and independent of caspase activation.[29,34–36,59,61,62] Further studies are required to integrate the requirement for caspases in MOMP induction.

Evidence from in vitro liposome studies, single-cell kinetic analysis and various genetic models has failed to provide overwhelming evidence that the OMM or the IMM directly regulates or induces MOMP. Thus, according to our innocent bystander hypothesis, the commitment to die in this pathway, or rather the commitment step that results in induction of MOMP rests entirely with the BCL-2 family of proteins and their regulators. We do not argue that the OMM or IMM does not participate in the induction of MOMP but that, if they do, they do so in a manner that always depends on the activities of the BH3-only or multidomain BCL-2 proteins in the cytosol. More importantly, the innocent bystander scenario proposes that MOMP can, and we suggest does, occur in the absence of decision-making events (proteins, membrane changes, ROS etc.) that are intrinsic to the mitochondrial membranes.

Acknowledgements

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