PROSPECTS
Journal of Cellular Biochemistry 115:632–640 (2014)

Journal of Cellular Biochemistry

Apoptosis Regulation at the Mitochondrial Outer Membrane

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ABSTRACT

Mitochondria play a critical role in apoptosis, or programmed cell death, by releasing apoptogenic factors from the intermembrane space. This process, known as mitochondrial outer membrane permeabilization (MOMP), is tightly regulated by the Bcl-2 family proteins. Pro-apoptotic Bcl-2 family members, Bax and Bak, change their conformation when activated by BH3 domain-only proteins in the family and permeabilize the MOM, whereas pro-survival members inhibit permeabilization. The precise nature of the apoptotic pore in the MOM is unknown, but is probably lipidic. Furthermore, it has been realized that there is another layer of MOMP regulation by a protein factor termed the catalyst in the MOM in order for Bax/Bak to achieve efficient and complete membrane permeabilization. Mitochondrial dynamics do not affect MOMP directly, but seem closely coordinated with MOMP for swift protein efflux from mitochondria. This review will present current views on the molecular mechanisms and regulation of MOMP and conclude with recent developments in clinical applications based on the knowledge gleaned from the investigation. J. Cell. Biochem. 115: 632–640, 2014.

KEY WORDS: APOPTOSIS; Bcl-2 FAMILY PROTEINS; MITOCHONDRIA

During development of a multicellular organism or in the homeostasis of the immune system, apoptosis eliminates unwanted cells. It is an active form of cell death, and unlike necrotic death, does not incite an inflammatory reaction, which may damage the surrounding tissues. During adulthood, apoptotic death serves to prevent proliferation of malignantly transformed cells by sensing intracellular aberration. Indeed, almost all cancer cells are defective in apoptosis [Hanahan and Weinberg, 2011], and tumorigenesis is enhanced greatly when apoptosis pathways are blocked [Vaux et al., 1988; Letai et al., 2004]. Thus, while inhibition of apoptosis alone may not be oncogenic, a growing body of evidence supports an important role for apoptosis in cancer biology. Understanding the molecular mechanisms of apoptosis will enable us to develop effective drugs that, by inducing cell death, thwart uncontrollable proliferation of malignant cells.

The genetic pathway of apoptosis, which is largely conserved in all multicellular organisms, was elucidated in *C. elegans* [Ellis et al., 1991]. Apoptosis culminates in the activation of cysteine-aspartic proteases (caspases) [Danial and Korsmeyer, 2004], and apoptotic cells exhibit similar morphological changes (shrinking, chromatin condensation, and blebbing of the plasma membrane) [Kerr et al., 1972], suggesting the existence of a common pathway. The mammalian homologues in the pathway have been identified [Yuan et al., 1993; Hengartner and Horvitz, 1994; Li et al., 1997; Zou et al., 1997], but a major twist in apoptosis in vertebrates is that it involves mitochondria [Newmeyer et al., 1994], which is not the case in *C. elegans* or *D. melanogaster* [Zimmermann et al., 2002]. It was a surprising discovery that, in vertebrates, cytochrome *c* that normally resides in the intermembrane space of mitochondria and participates in oxidative phosphorylation induced caspase activation in the cytosol [Liu et al., 1996; Kluck et al., 1997b]. Subsequently, it was revealed that mitochondrial outer membranes permeabilize to release cytochrome *c* and other intermembrane space proteins [Du et al., 2000; Verhagen et al., 2000; Martins et al., 2002; Cilenti et al., 2003; Yang et al., 2003] that also facilitate caspase activation. The permeabilization of the mitochondrial outer membrane in response to apoptotic signals and the subsequent release of apoptogenic factors represent a point of no return in the apoptosis pathway; once mitochondrial outer membrane permeabilization (MOMP) occurs, cells do not recover due to defective mitochondrial function [Chipuk and Green, 2005; Lartigue et al., 2009]. When Bcl-2 family proteins were found to regulate this membrane permeabilization process [Kluck et al., 1997a; Yang et al., 1997], regulation of apoptosis at mitochondria emerged as a great opportunity for intervention in diseases such as cancer and became the focus of intensive studies.

Grant sponsor: NIH.
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Manuscript Received: 1 October 2013; Manuscript Accepted: 31 October 2013
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 7 November 2013
DOI 10.1002/jcb.24709 • © 2013 Wiley Periodicals, Inc.

BCL-2 FAMILY PROTEINS ARE CENTRAL TO APOPTOSIS REGULATION AT THE MOM

BCL-2 (B-cell lymphoma 2) was first discovered in B-cell follicular lymphoma as an "oncogene" that is translocated to the locus under the control of the immunoglobulin heavy chain gene promoter. The resulting over-expression of Bcl-2 protein in these cells inhibits cell death and promotes carcinogenesis [Bakhshi et al., 1985; Tsujimoto et al., 1985; Cleary et al., 1986; Vaux et al., 1988]. Subsequently, 20 or so Bcl-2 family members were identified [Kuwana and Newmeyer, 2003; Youle and Strasser, 2008; Chipuk et al., 2010]. Bcl-2 family proteins share up to four Bcl-2 homology domains (BH1-4) and they are divided into pro-apoptotic and pro-survival subsets. The pro-apoptotic members consist of multi-domain and BH3-domain only proteins and the latter group has de-repressors and activators (Fig. 1). MOMP was recapitulated in simplified systems that utilize isolated outer membrane vesicles (OMVs) and recombinant Bcl-2 family proteins, indicating that the primary site for the function of Bcl-2 family proteins is at the MOM, regulating its permeabilization. In other words, the cell fate is decided at the MOM [Kuwana et al., 2002]. The membrane permeabilizing activity of Bcl-2 family proteins has been reconstituted in artificial lipid membranes [Kuwana et al., 2002; Terrones et al., 2004; Lovell et al., 2008], implicating these proteins as direct effectors of MOMP.

Bcl-2 family proteins interact with one another primarily through their BH3 domains. The current model posits that select BH3-only proteins (activator BH3-only proteins) activate Bax and Bak directly [Letai et al., 2002; Kuwana et al., 2005] through a transient ("hit and run") interaction. For example, the caspase-cleaved, active form of Bid protein can activate Bax to permeabilize liposome membranes, even though a stable complex of Bax and Bid is not detectable. On the other hand, pro-survival proteins inhibit this process by sequestering BH3-only proteins and/or Bax/Bak [Chen et al., 2005; Czabotar et al., 2007; Billen et al., 2008; Du et al., 2011; Llambi et al., 2011]. In order for MOMP to occur, two events must take place: (1) All the pro-survival members of the Bcl-2 family are inhibited and (2) Bax or Bak is activated by the activator BH3-only proteins. In other words, thorough inhibition of pro-survival Bcl-2 family proteins (de-repression/sensitization) is not sufficient to promote membrane permeabilization; rather, direct activation of Bax or Bak is required [Du et al., 2011]. De-repressor/sensitizer BH3-only proteins inhibit pro-survival Bcl-2 family proteins, but cannot activate Bax and Bak directly, whereas activator BH3-only proteins can do both. There is some controversy over which BH3-only proteins are direct activators apart from Bim and Bid. If all the direct activators are absent, the cells are expected to be resistant to apoptosis like Bax/Bak double knockout cells. Bim/Bid/Puma triple knockout mice were generated to test this hypothesis. These mice showed a similar phenotype to Bax/Bak double knockout species, showing the interdigital web and unperforated vagina [Ren et al., 2010]. However, some raised a concern that the phenotype was not as profound as that of Bax/Bak double knockout mice [Villunger et al., 2011]. Bax and Bak might be activated by some other means: p53 [Chipuk et al., 2004] and heat treatment [Pagliari et al., 2005] are known to activate Bax/Bak directly, and this might be the reason for the less profound phenotype. Another possibility is that other BH3-only proteins act like direct activators. We observed that BH3 peptides other than Bim and Bid, such as Puma, Bmf, and Noxa (Fig. 1), showed modest, but reproducible, direct activation [Du et al., 2011]. We speculate that direct activation is graded and not all-or-none.

BAX/BAK CONFORMATIONAL CHANGES LEADING TO MOMP

All the multi-domain Bcl-2 family proteins have the same folded structure in solution: neatly packed α-helices with hydrophobic residues, facing the inside of the molecule [Muchmore et al., 1996; Suzuki et al., 2000]. Their primary site of action is in the membrane, and yet the structure for membrane-engaged Bcl-2 family proteins is unknown [Leber et al., 2007]. Due to the technical difficulty in developing in vitro systems with membrane proteins, researchers have addressed mechanistic questions using truncated Bcl-2 family proteins and by solving solution structures. Bax and Bak are functionally redundant and, upon activation, undergo similar conformational change and oligomerization in the MOM [Griffiths et al., 1999; Hsu and Youle, 1997]. However, Bax is mostly localized in the cytoplasm in healthy cells and translocates to mitochondria during apoptosis [Wolter et al., 1997], whereas Bak constitutively resides in the MOM. When the full-length Bax NMR structure was solved, it was found that the BH3 domain constitutes part of a hydrophobic groove, in which the hydrophobic C-terminal helix is accommodated [Suzuki et al., 2000] (Fig. 2a). When an activator BH3-only protein interacts with the trigger site on Bax transiently through the 'hit-and-run' mechanism [Wei et al., 2000; Gavathiotis et al., 2008], Bax unfolds its helices and inserts the C-terminus into the membrane [Gavathiotis et al., 2010](Fig. 2b). The initiation of direct activation observed in Bax through the trigger site might be unique to Bax, as Bak is already in the membrane. Guided by truncated Bak structure [Moldoveanu et al., 2006] and intramolecular cross-linking, Kluck and colleagues mapped the conformational changes taking place during Bak activation. They found that the BH3 domain of Bak becomes exposed and occupies the emptied hydrophobic groove of another Bak molecule that has also been activated. This BH3:groove interaction results in the formation of a symmetrical dimer, which then initiates subsequent dimer:dimer interaction through the opposite side of the molecule to form higher-order oligomers [Dewson et al., 2008, 2009](Fig. 2c). Further, it has been demonstrated that the BH3 domain of an activator BH3-only protein interacts with the Bak BH3-binding groove transiently and induces a conformational change that allows the BH3 domain of the activating protein to be displaced by another BH3 domain (in the physiological situation, presumably the BH3 domain of another Bak molecule) [Leshchiner et al., 2013; Moldoveanu et al., 2013].

The same theme has been extended to Bax [Dewson et al., 2012; Czabotar et al., 2013] and a unified model of Bcl-2 family protein interactions has emerged, in which all Bcl-2 family proteins interact through their BH3 domains and hydrophobic BH3 grooves. However, as opposed to what happens with Bax and Bak, BH3-domain binding to pro-survival proteins would lead to unproductive dimers [Dewson and Kluck, 2009]. Indeed, pro-survival Bcl-xL has been shown to bind both to a BH3-only protein, cBid, and Bax in the membrane, to

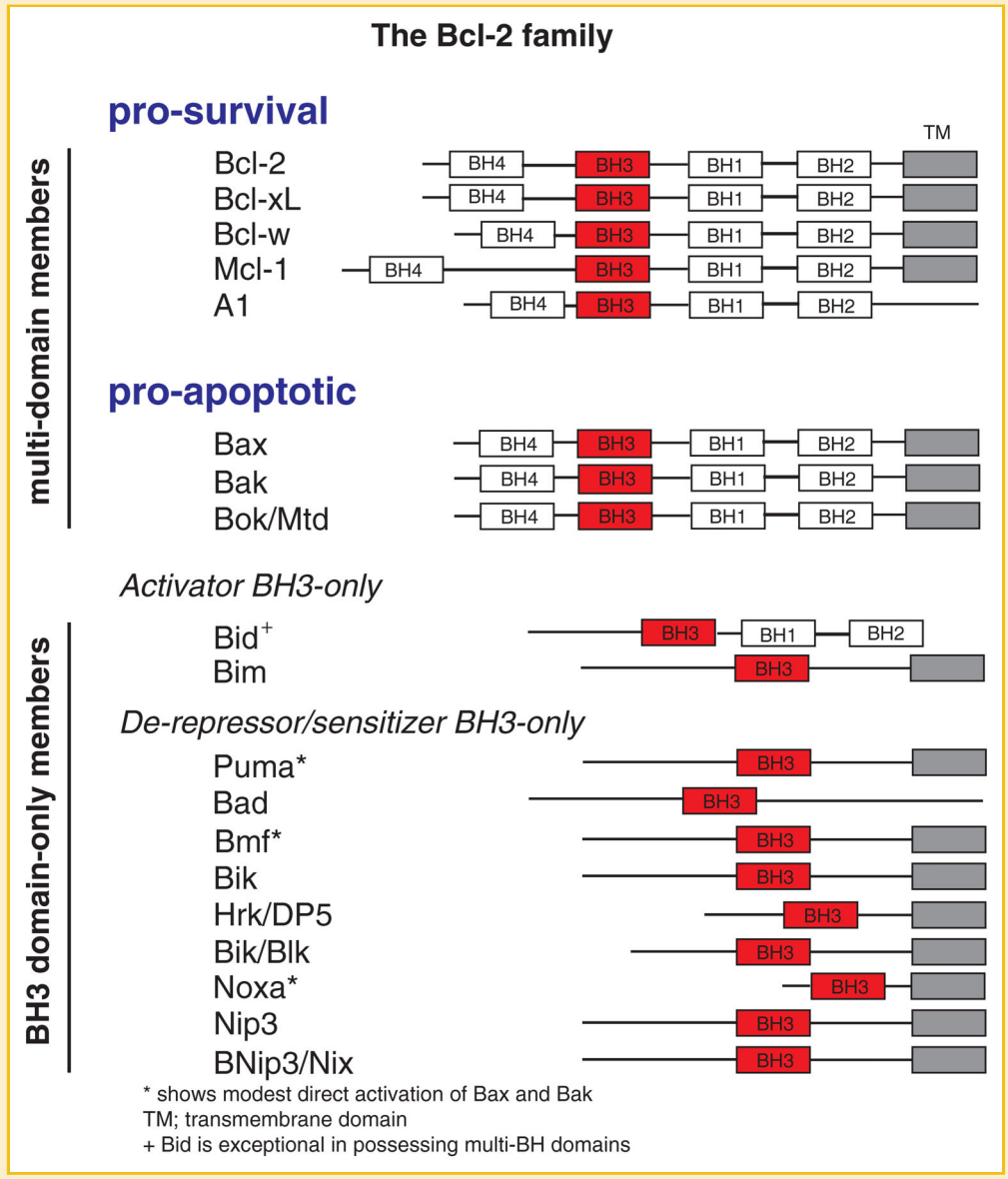

Fig. 1. The Bcl-2 family proteins. The Bcl-2 family proteins regulate apoptosis at the mitochondrial outer membrane. They share up to four Bcl-2 homology (BH) domains, of which the BH3 domain is functionally critical. They are divided into pro-survival and—apoptotic categories. There are pro-apoptotic members possessing the BH3 domain ony (BH3-only proteins) in the pro-apoptotic category. They inhibit pro-survival members, but some BH3-only proteins can activate Bax and Bak directly (activator BH3-only).

prevent Bax from permeabilizing the membrane and from oligomer formation [Billen et al., 2008] (Fig. 2d).

Confocal and immunoelectron microscopy showed that Bax accumulates in foci on mitochondria [Nechushtan et al., 2001], suggesting that Bax oligomers may be functionally important during apoptosis. Indeed, Bax oligomerization and MOMP appear to be tightly coupled events [Antonsson et al., 2000; Saito et al., 2000; Wei et al., 2000; George et al., 2007; Dewson et al., 2008; Dewson et al., 2009; Czabotar et al., 2013]; that is, Bax mutants that do not oligomerize, invariably fail to induce MOMP and apoptosis. However, these mutational studies cannot distinguish oligomerization from other defects in unfolding prior to oligomer formation, and therefore cannot prove that oligomers are a prerequisite for MOMP. Although oligomerization could in theory increase the membrane stress induced by Bax, it could also be that it is merely be a side-effect of Bax unfolding and membrane integration. Using an in vitro liposome system and fluorescently labeled proteins, Andrews and colleagues showed that Bax was oligomerized into the membrane continually even after the membrane was permeabilized, questioning the causal relationship between large Bax oligomers and MOMP [Lovell et al., 2008]. A recent study showed that the kinetics of dextran release from OMVs displayed no cooperativity with respect to Bax, suggesting that Bax oligomerization may not be required for membrane permeabilization, or oligomerization does not dictate the kinetics of membrane permeabilization [Kushnareva et al., 2012]. Instead, it is predicted by mathematical modeling that the

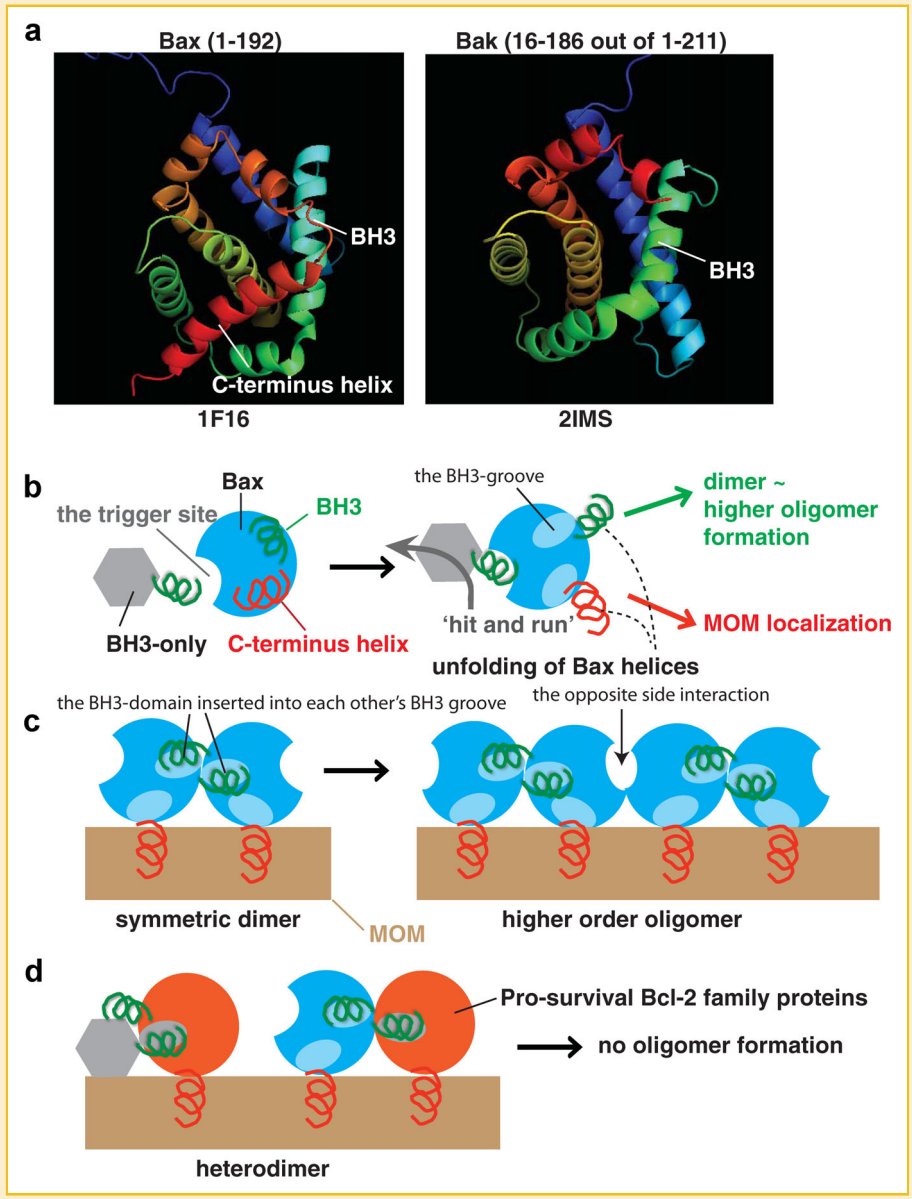

Fig. 2. Bax/Bak activation and their interactions with other Bcl-2 family members. (a) Full-length Bax NMR structure (1F16; PDB) [Suzuki et al., 2000] and truncated Bak crystal structure (2IMS; PDB) [Moldoveanu et al., 2006]. The molecules are rainbow color-coded from the N-terminus (blue) to the C-terminus (red). Note the same helical packing. (b) Bax activation is initiated at the trigger site on the molecule by an activator BH3-only protein. This interaction unfolds the C-terminus helix and exposes the BH3 domain, which leads to the MOM translocation and dimer to higher order oligomer formation, respectively. (c) Bax or Bak homodimers interact with the side of the molecule opposite to the BH3-groove to form higher order oligomers. (d) Pro-survival proteins inhibit MOMP by sequestering BH3-only proteins as well as binding to activated Bax/Bak in the membrane. Both heterodimers are unproductive for membrane permeabilization.

oligomerization of an unknown molecule (the catalyst) takes place prior to MOMP. Another provocative report showed that a single activated Bax molecule could generate a ~3.5 nm pore in a nanodisc lipid bilayer [Xu et al., 2013]. This report clearly shows that Bax does not have to be oligomerized to permeabilize the lipid bilayer. This monomeric Bax-induced pore could be the initial site of nucleation for the larger Bax-induced pore observed in liposomes (see Membrane permeabilization below) [Schafer et al., 2009]. A hypothetical mechanism of pore enlargement could involve either the coalescence of several monomeric Bax pores or the joining of additional Bax molecules to an existing pore.

One can address mechanistic questions in liposome systems that are not possible in more complex systems. However, liposomes do not reproduce the complexities of Bax-induced membrane permeabilization in the native MOM. Indeed, the kinetics of dextran release from OMVs revealed a prolonged lag phase prior to the release (presumably the period when the catalyst molecules are assembling), which is not seen in the release curves in liposomes [Kushnareva

et al., 2012]. Further studies will be needed to determine what components in the MOM co-operate with activated Bax to achieve membrane permeabilization.

MEMBRANE PERMEABILIZATION

A great deal of study has revealed the conformational changes of Bax and Bak during MOMP. However, questions remain regarding what kind of "pores" would result from such changes of Bax and Bak that allow the efflux of macromolecules. We investigated MOM pore characteristics utilizing OMVs loaded with fluorescein-conjugated dextrans of different sizes, in order to determine the size of Bax-induced pores. To our surprise, 10 kD (Stokes diameter; 5 nm) and 2,000 kD (Stokes diameter; 50 nm) dextrans were both released almost completely from these vesicles upon incubation with recombinant Bax and an activator BH3-only protein (cBid) [Kuwana et al., 2002]. However, we did not detect any pore-like structures by conventional thin section or negative stain electron microscopy (EM), suggesting that the pores may be delicate structures that are not preserved by the standard fixation and staining procedures for EM. However, in later studies, we and others visualized Bax-induced pore structures in liposomes using cryo-EM, which captures the membranes in their native and hydrated state [Schafer et al., 2009; Bleicken et al., 2010; Landeta et al., 2011]. Morphologically, these Bax-induced pores are clearly different from proteinaceous pores [Tilley et al., 2005]. They do not show the macromolecular structures around the pore edges like the proteinaceous pores do. Instead, the edges of the Bax-induced pores exhibit the negative (inward) curvature, suggesting the involvement of curvature stress as a pore formation mechanism. Together with the observation that phospholipids translocate from one leaflet to the other in Bax permeabilized liposomes [Terrones et al., 2004], we conclude that Bax pores fit the toroidal (or lipidic) pore (α-pore) model, as opposed to the totally proteineceous barrel-stave pore (β-pore) model [Tilley and Saibil, 2006] (Fig. 3). Of note, the structures of multi-domain Bcl-2 family proteins including Bax and Bak resemble those of diphtheria toxin and colicin, which form α-pores [Muchmore et al., 1996; Suzuki et al., 2000; Moldoveanu et al., 2006]. Bax-induced pores in the native MOM remain to be visualized.

An alternative pore model arose from researchers measuring patch-clamp channel activity. Such analysis revealed a high-conductance channel in apoptotic mitochondria, termed MAC (mitochondrial apoptosis-inducing channel) [Pavlov et al., 2001; Guihard et al., 2004]. It was also found that oligomeric Bax or Bak constituted the MAC. MAC was proposed to be formed from oligomeric Bax or Bak [Dejean et al., 2005] and shown to increase in size up to ~6 nm in diameter [Martinez-Caballero et al., 2009]. Furthermore, MAC was hypothesized to be a barrel-stave pore because of its step-wise increase in conductance, although this observation does not preclude the idea of the toroidal pore increasing in size when Bax molecules are added one by one. There is a large discrepancy between the predicted 6 nm size of MAC and the functional sizes observed for Bax-induced pores in OMVs: over ~50 nm. Mitochondria release a variety of intermembrane space proteins at the same rate [Munoz-Pinedo et al., 2006], including 12 kD cytochrome *c* (Stokes diameter; 3.3 nm [Erickson, 2009]) and

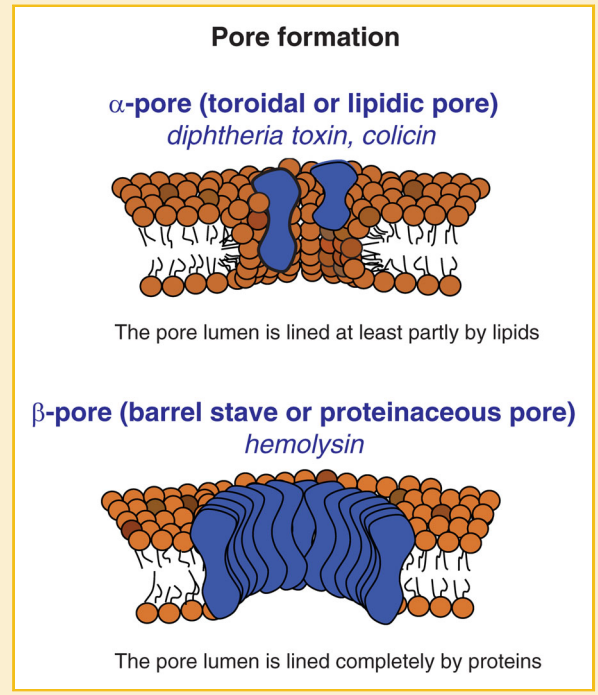

Fig. 3. Two types of pores formed by polypeptides. The α-pore (toroidal or lipidic pore) is presumed to be formed by diphtheria toxin, colicin, or Cry toxins. These toxins are thought to unfold their helices before generating a hole in their target membrane. Some proteins possess helices too short to span the entire bilayer. It is hypothesized that these proteins exert a detergent-like action, including the induction of high curvature in the bilayer, which would lead to the pore whose lumen consists partly of lipids. Multi-domain Bcl-2 family proteins possess the same helical arrangements as those of diphtheria toxin and clicin. On the other hand, the β-pore (barrel-stave or proteinaceous pore) is constituted with oligomerized toxin molecules lining the lumen of the pore.

tetramers of 25 kD SMAC (Stokes diameter; 6 nm) [Du et al., 2000]. In principle, a 6 nm pore should be sufficient even for the release of SMAC tetramers. In a proteomic study [Van Loo et al., 2002], the largest polypeptides released from mitochondria were the type-I RNA helicase (123 kD) and endoplasmin (93 kD). It is unknown whether any intermembrane space proteins exist as larger multimeric species, But if they exist, they may readily be released from mitochondria after MOMP, given the observed release of huge 2,000 kD dextrans through Bax-induced pores in OMVs.

MITOCHONDRIAL DYNAMICS AND MOMP

Mitochondria are in dynamic equilibrium, in that they continually fuse and divide, and this is necessary for them to maintain their function [Westermann, 2010; Youle and van der Bliek, 2012]. There are a number of molecules identified that orchestrate mitochondrial fusion or fission. It was a striking observation that mitochondria fragment during apoptosis [Frank et al., 2001]. However, further studies reported that MOMP and mitochondrial dynamics can be uncoupled in certain contexts [Autret and Martin, 2009; Delivani et al., 2006; Parone et al., 2006; Sheridan et al., 2008]. Therefore, mitochondrial fission during apoptosis is not an absolute requirement

for MOMP. Over-expression of a mammalian fission protein, Drp-1 (dynamin related protein-1), induces gross morphological alterations to mitochondrial cristae, and the outer and inner membranes are torn apart [Cribbs and Strack, 2007]. With such disorganized cristae, the release of the intermembrane space proteins would most likely be altered, even though MOMP itself is not affected.

Nunnari and colleagues screened and identified chemical compounds (analogues of mitochondrial division inhibitor-1, or mdivi-1) that inhibit mitochondrial fission in yeast through their inhibition of GTPase activity of Dnm-1 (yeast homologue of Drp-1). [Cassidy-Stone et al., 2008]. They subsequently showed that certain analogues of mdivi-1 delayed cytochrome *c* release in mammalian cells. However, it also inhibited cytochrome *c* release from isolated mitochondria that do not undergo fission during MOMP, suggesting that mdivi-1′s inhibition of MOMP is not mediated through mitochondrial fission *per se*. It might be that Drp-1 promotes MOMP by a different activity [Montessuit et al., 2010], or that mdivi-1′s target is not Drp-1. A recent report found that mdivi-1 analogs do inhibit MOMP in a system using OMVs [Kushnareva et al., 2012], apparently consistent with the original report. However, Drp-1 (normally localized in the cytoplasm) was not detected by immunoblot in these OMVs, casting doubt on Drp-1 as the sole target of mdivi-1.

Some proteins involved in mitochondrial dynamics, such as Drp-1 and a mitochondrial fusion protein, Mfn-2, are colocalized with Bax in apoptotic mitochondria [Karbowski et al., 2002] and stabilization of Drp-1 in mitochondrial membranes (which would appear as Drp-1 recruitment to mitochondria) is Bax/Bak dependent [Wasiak et al., 2007]. Taken together, these studies imply that there is a close coordination between MOMP and mitochondrial dynamics, although the molecular mechanisms are unknown. The problems associated with studying the roles these proteins play in MOMP are; first, perturbing mitochondrial dynamics could stress cells and activate apoptotic signaling, leading to the interpretation that they are pro-apoptotic and second, disorganization of mitochondrial membrane structure resulting from perturbing mitochondrial dynamics might lead to altered protein release from the intermembrane space, appearing as if MOMP is altered. It is important to separate effects of these proteins on MOMP from those on whole mitochondria.

THERAPEUTIC IMPLICATIONS

The BH3 domains of the BH3-only proteins were proposed to work like ligands for one another [Wei et al., 2000], and even short peptides corresponding to the BH3 domains of these proteins mimicked the functions of full-length proteins [Chen et al., 2005; Cosulich et al., 1997; Kuwana et al., 2005; Letai et al., 2002]. In many forms of cancer, pro-survival proteins are upregulated and promote proliferation of malignant cells and/or confer resistance to chemotherapy [Adams and Cory, 2007]. Therefore, inhibition of pro-survival Bcl-2 proteins is considered a promising avenue for cancer therapy. One of the logical approaches is identify compounds that mimic the effect of BH3 peptides in inhibiting pro-survival proteins. The first BH3-mimetic compound, ABT-737, was discovered through NMR-based screening of a chemical library followed by structure-based design [Oltersdorf et al., 2005]. ABT-737 has proven to be effective in reducing the tumor burden in mouse tumor models [Del Gaizo Moore et al., 2007; Kutuk and Letai, 2008]. However, inhibition of Mcl-1 and A1 by ABT-737 is weak, and this often poses a problem in that some hematopoietic malignancies express high levels of Mcl-1 [Konopleva et al., 2006; van Delft et al., 2006]. Other BH3 mimetics have been developed to compensate for this defect, specifically targeting Mcl-1 [Verhaegen et al., 2006; Nguyen et al., 2007; Dash et al., 2011]. However, more detailed investigation has found that these compounds often induce cell death through mechanisms other than Bax/Bak-mediated MOMP: they kill cells independent of Bax/Bak and/or caspases [Vogler et al., 2009a,b]. It is necessary to further refine the BH3 mimetics by structure-based design in order to develop more specific reagents, reducing off-target, and side effects. A number of BH3 mimetics, or small molecule inhibitors of pro-survival Bcl-2 family proteins have been reported and some of them are in phase II clinical trials [Azmi and Mohammad, 2009; Elkholi et al., 2011].

Letai and colleagues introduced the concept of "BH3 profiling," which is a way to decode the susceptibility of mitochondria to MOMP in response to apoptotic signaling [Certo et al., 2006]. They examined MOMP in isolated mitochondria by a panel of BH3 peptides and found that mitochondria in transformed or stressed cells are ready to undergo MOMP upon inhibition of pro-survival Bcl-2 family proteins and those in primary cells are not [Certo et al., 2006; Ni Chonghaile et al., 2011]. These mitochondria harbor pro-survival Bcl-2 proteins bound to activator BH3-only proteins. Therefore, de-repressor BH3 peptides will release the activator BH3-only proteins from the pro-survival proteins, and the released activator BH3-onlys will in turn activate Bax/Bak and induce MOMP. They termed such mitochondria "primed." When mitochondria are not primed, that is, their pro-survival proteins are not associated with activator BH3-only proteins, cells are resistant to de-repressor reagents like ABT-737, and mobilization of activator BH3-only proteins by other cytotoxic drugs is required to induce cell death. Subsequently, an assay to probe mitochondrial priming was developed. It was found that, in leukemia patients, highly primed mitochondria predicted the patients′ positive response to chemotherapy and good prognosis [Ni Chonghaile et al., 2011; Davids et al., 2012; Vo et al., 2012]. Future challenges include extending the significance of mitochondrial priming to solid tumors and coupling it with BH3 mimetic drugs for targeted treatment.

Although mitochondrial priming does not reveal the precise make-up of Bcl-2 family proteins on mitochondria, it has been shown to be valuable for predicting patient outcomes, so that physicians can determine the most beneficial therapeutic strategies. Many challenges remain in understanding the regulation of MOMP, such as investigating the roles of auxiliary proteins (those not belonging to the Bcl-2 family); however, we anticipate that Bcl-2 family proteins will remain central to MOMP regulation, and many effective therapeutic interventions will come from knowledge and manipulation of these proteins.

ACKNOWLEDGMENT

We thank Dr Donald Newmeyer for stimulating discussion and critical reading of the manuscript.

REFERENCES

Adams JM, Cory S. 2007. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337.

Antonsson B, Montessuit S, Lauper S, Eskes R, Martinou JC. 2000. Bax oligomerization is required for channel-forming activity in liposomes and to trigger cytochrome c release from mitochondria. Biochem J 345 Pt 2:271–278.

Autret A, Martin SJ. 2009. Emerging role for members of the Bcl-2 family in mitochondrial morphogenesis. Mol Cell 36:355–363.

Azmi AS, Mohammad RM. 2009. Non-peptidic small molecule inhibitors against Bcl-2 for cancer therapy. J Cell Physiol 218:13–21.

Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride OW, Epstein AL, Korsmeyer SJ. 1985. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: Clustering around JH on chromosome 14 and near a transcriptional unit on 18. Cell 41:899–906.

Billen LP, Kokoski CL, Lovell JF, Leber B, Andrews DW. 2008. Bcl-XL inhibits membrane permeabilization by competing with Bax. PLoS Biol 6:e147.

Bleicken S, Classen M, Padmavathi PV, Ishikawa T, Zeth K, Steinhoff HJ, Bordignon E. 2010. Molecular details of Bax activation, oligomerization, and membrane insertion. J Biol Chem 285:6636–6647.

Cassidy-Stone A, Chipuk JE, Ingerman E, Song C, Yoo C, Kuwana T, Kurth MJ, Shaw JT, Hinshaw JE, Green DR, Nunnari J. 2008. Chemical inhibition of the mitochondrial division dynamin reveals its role in bax/bak-dependent mitochondrial outer membrane permeabilization. Dev Cell 14:193–204.

Certo M, Del Gaizo Moore V, Nishino M, Wei G, Korsmeyer S, Armstrong SA, Letai A. 2006. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell 9:351–365.

Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, Colman PM, Day CL, Adams JM, Huang DC. 2005. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell 17:393–403.

Chipuk JE, Green DR. 2005. Do inducers of apoptosis trigger caspase-independent cell death? Nature reviews. Mol Cell Biol 6:268–275.

Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M, Green DR. 2004. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science 303:1010–1014.

Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR. 2010. The BCL-2 family reunion. Mol Cell 37:299–310.

Cilenti L, Lee Y, Hess S, Srinivasula S, Park KM, Junqueira D, Davis H, Bonventre JV, Alnemri ES, Zervos AS. 2003. Characterization of a novel and specific inhibitor for the pro-apoptotic protease Omi/HtrA2. J Biol Chem 278:11489–11494.

Cleary ML, Smith SD, Sklar J. 1986. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell 47:19–28.

Cosulich SC, Worrall V, Hedge PJ, Green S, Clarke PR. 1997. Regulation of apoptosis by BH3 domains in a cell-free system. Curr Biol 7:913–920.

Cribbs JT, Strack S. 2007. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. EMBO Rep 8:939–944.

Czabotar PE, Lee EF, van Delft MF, Day CL, Smith BJ, Huang DC, Fairlie WD, Hinds MG, Colman PM. 2007. Structural insights into the degradation of Mcl-1 induced by BH3 domains. Proc Natl Acad Sci USA 104:6217–6222.

Czabotar PE, Westphal D, Dewson G, Ma S, Hockings C, Fairlie WD, Lee EF, Yao S, Robin AY, Smith BJ, Huang DC, Kluck RM, Adams JM, Colman PM. 2013. Bax crystal structures reveal how BH3 domains activate Bax and nucleate its oligomerization to induce apoptosis. Cell 152:519–531.

Danial NN, Korsmeyer SJ. 2004. Cell death: Critical control points. Cell 116:205–219.

Dash R, Azab B, Quinn BA, Shen X, Wang XY, Das SK, Rahmani M, Wei J, Hedvat M, Dent P, Dmitriev IP, Curiel DT, Grant S, Wu B, Stebbins JL, Pellecchia M, Reed JC, Sarkar D, Fisher PB. 2011. Apogossypol derivative BI-97C1 (Sabutoclax) targeting Mcl-1 sensitizes prostate cancer cells to mda-7/IL-24-mediated toxicity. Proc Natl Acad Sci USA 108:8785–8790.

Davids MS, Deng J, Wiestner A, Lannutti BJ, Wang L, Wu CJ, Wilson WH, Brown JR, Letai A. 2012. Decreased mitochondrial apoptotic priming underlies stroma-mediated treatment resistance in chronic lymphocytic leukemia. Blood 120:3501–3509.

Dejean LM, Martinez-Caballero S, Guo L, Hughes C, Teijido O, Ducret T, Ichas F, Korsmeyer SJ, Antonsson B, Jonas EA, Kinnally KW. 2005. Oligomeric Bax is a component of the putative cytochrome c release channel MAC, mitochondrial apoptosis-induced channel. Mol Biol Cell 16:2424–2432.

Del Gaizo Moore V, Brown JR, Certo M, Love TM, Novina CD, Letai A. 2007. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. J Clin Invest 117:112–121.

Delivani P, Adrain C, Taylor RC, Duriez PJ, Martin SJ. 2006. Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. Mol Cell 21:761–773.

Dewson G, Kluck RM. 2009. Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis. J Cell Sci 122:2801–2808.

Dewson G, Kratina T, Sim HW, Puthalakath H, Adams JM, Colman PM, Kluck RM. 2008. To trigger apoptosis, Bak exposes its BH3 domain and homodimerizes via BH3:groove interactions. Mol Cell 30:369–380.

Dewson G, Kratina T, Czabotar P, Day CL, Adams JM, Kluck RM. 2009. Bak activation for apoptosis involves oligomerization of dimers via their alpha6 helices. Mol Cell 36:696–703.

Dewson G, Ma S, Frederick P, Hockings C, Tan I, Kratina T, Kluck RM. 2012. Bax dimerizes via a symmetric BH3:groove interface during apoptosis. Cell Death Differ 19:661–670.

Du C, Fang M, Li Y, Li L, Wang X. 2000. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 102:33–42.

Du H, Wolf J, Schafer B, Moldoveanu T, Chipuk JE, Kuwana T. 2011. BH3 domains other than Bim and Bid can directly activate Bax/Bak. J Biol Chem 286:491–501.

Elkholi R, Floros KV, Chipuk JE. 2011. The role of BH3-only proteins in tumor cell development, signaling, and treatment. Genes Cancer 2:523–537.

Ellis RE, Yuan JY, Horvitz HR. 1991. Mechanisms and functions of cell death. Annu Rev Cell Biol 7:663–698.

Erickson HP. 2009. Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. Biol Proc Online 11:32–51.

Frank S, Gaume B, Bergmann-Leitner ES, Leitner WW, Robert EG, Catez F, Smith CL, Youle RJ. 2001. The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev Cell 1:515–525.

Gavathiotis E, Suzuki M, Davis ML, Pitter K, Bird GH, Katz SG, Tu HC, Kim H, Cheng EH, Tjandra N, Walensky LD. 2008. BAX activation is initiated at a novel interaction site. Nature 455:1076–1081.

Gavathiotis E, Reyna DE, Davis ML, Bird GH, Walensky LD. 2010. BH3-triggered structural reorganization drives the activation of proapoptotic BAX. Mol Cell 40:481–492.

George NM, Evans JJ, Luo X. 2007. A three-helix homo-oligomerization domain containing BH3 and BH1 is responsible for the apoptotic activity of Bax. Genes Dev 21:1937–1948.

Griffiths GJ, Dubrez L, Morgan CP, Jones NA, Whitehouse J, Corfe BM, Dive C, Hickman JA. 1999. Cell damage-induced conformational changes of the pro-apoptotic protein Bak in vivo precede the onset of apoptosis. J Cell Biol 144:903–914.

Guihard G, Bellot G, Moreau C, Pradal G, Ferry N, Thomy R, Fichet P, Meflah K, Vallette FM. 2004. The mitochondrial apoptosis-induced channel (MAC) corresponds to a late apoptotic event. J Biol Chem 279:46542–46550.

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: The next generation. Cell 144:646–674.

Hengartner MO, Horvitz HR. 1994. C. elegans cell survival gene ced-9 encodes a functional homolog of the mammalian proto-oncogene bcl-2. Cell 76:665–676.

Hsu YT, Youle RJ. 1997. Nonionic detergents induce dimerization among members of the Bcl-2 family. J Biol Chem 272:13829–13834.

Karbowski M, Lee YJ, Gaume B, Jeong SY, Frank S, Nechushtan A, Santel A, Fuller M, Smith CL, Youle RJ. 2002. Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. J Cell Biol 159:931–938.

Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257.

Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. 1997a. The release of cytochrome c from mitochondria: A primary site for Bcl-2 regulation of apoptosis. Science 275:1132–1136.

Kluck RM, Martin SJ, Hoffman BM, Zhou JS, Green DR, Newmeyer DD. 1997b. Cytochrome c activation of CPP32-like proteolysis plays a critical role in a Xenopus cell-free apoptosis system. Embo J 16:4639–4649.

Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi YX, Sneed T, Verhaegen M, Soengas M, Ruvolo VR, McQueen T, Schober WD, Watt JC, Jiffar T, Ling X, Marini FC, Harris D, Dietrich M, Estrov Z, McCubrey J, May WS, Reed JC, Andreeff M. 2006. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 10:375–388.

Kushnareva Y, Andreyev AY, Kuwana T, Newmeyer DD. 2012. Bax activation initiates the assembly of a multimeric catalyst that facilitates bax pore formation in mitochondrial outer membranes. PLoS Biol 10:e1001394.

Kutuk O, Letai A. 2008. Alteration of the mitochondrial apoptotic pathway is key to acquired paclitaxel resistance and can be reversed by ABT-737. Cancer Res 68:7985–7994.

Kuwana T, Newmeyer DD. 2003. Bcl-2-family proteins and the role of mitochondria in apoptosis. Curr Opin Cell Biol 15:691–699.

Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR, Newmeyer DD. 2002. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 111:331–342.

Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR, Newmeyer DD. 2005. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol Cell 17:525–535.

Landeta O, Landajuela A, Gil D, Taneva S, Di Primo C, Sot B, Valle M, Frolov VA, Basanez G. 2011. Reconstitution of proapoptotic BAK function in liposomes reveals a dual role for mitochondrial lipids in the BAK-driven membrane permeabilization process. J Biol Chem 286:8213–8230.

Lartigue L, Kushnareva Y, Seong Y, Lin H, Faustin B, Newmeyer DD. 2009. Caspase-independent mitochondrial cell death results from loss of respiration, not cytotoxic protein release. Mol Biol Cell 20:4871–4884.

Leber B, Lin J, Andrews DW. 2007. Embedded together: The life and death consequences of interaction of the Bcl-2 family with membranes. Apoptosis 12:897–911.

Leshchiner ES, Braun CR, Bird GH, Walensky LD. 2013. Direct activation of full-length proapoptotic BAK. Proc Natl Acad Sci USA 110:E986–E995.

Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. 2002. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell 2:183–192.

Letai A, Sorcinelli MD, Beard C, Korsmeyer SJ. 2004. Antiapoptotic BCL-2 is required for maintenance of a model leukemia. Cancer Cell 6:241–249.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91:479–489.

Liu X, Kim CN, Yang J, Jemmerson R, Wang X. 1996. Induction of apoptotic program in cell-free extracts: Requirement for dATP and cytochrome c. Cell 86:147–157.

Llambi F, Moldoveanu T, Tait SW, Bouchier-Hayes L, Temirov J, McCormick LL, Dillon CP, Green DR. 2011. A unified model of mammalian BCL-2 protein family interactions at the mitochondria. Mol Cell 44:517–531.

Lovell JF, Billen LP, Bindner S, Shamas-Din A, Fradin C, Leber B, Andrews DW. 2008. Membrane binding by tBid initiates an ordered series of events culminating in membrane permeabilization by Bax. Cell 135:1074–1084.

Martinez-Caballero S, Dejean LM, Kinnally MS, Oh KJ, Mannella CA, Kinnally KW. 2009. Assembly of the mitochondrial apoptosis-induced channel, MAC. J Biol Chem 284:12235–12245.

Martins LM, Iaccarino I, Tenev T, Gschmeissner S, Totty NF, Lemoine NR, Savopoulos J, Gray CW, Creasy CL, Dingwall C, Downward J. 2002. The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a reaper-like motif. J Biol Chem 277:439–444.

Moldoveanu T, Liu Q, Tocilj A, Watson M, Shore G, Gehring K. 2006. The X-ray structure of a BAK homodimer reveals an inhibitory zinc binding site. Mol Cell 24:677–688.

Moldoveanu T, Grace CR, Llambi F, Nourse A, Fitzgerald P, Gehring K, Kriwacki RW, Green DR. 2013. BID-induced structural changes in BAK promote apoptosis. Nat Struct Mol Biol 20:589–597.

Montessuit S, Somasekharan SP, Terrones O, Lucken-Ardjomande S, Herzig S, Schwarzenbacher R, Manstein DJ, Bossy-Wetzel E, Basanez G, Meda P, Martinou JC. 2010. Membrane remodeling induced by the dynamin-related protein Drp1 stimulates Bax oligomerization. Cell 142:889–901.

Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettesheim D, Chang BS, Thompson CB, Wong S-L, Ng S-C, Fesik SW. 1996. X-ray and NMR structure of human Bcl-xL, an inhibityor of programmed cell death. Nature 381:335–341.

Munoz-Pinedo C, Guio-Carrion A, Goldstein JC, Fitzgerald P, Newmeyer DD, Green DR. 2006. Different mitochondrial intermembrane space proteins are released during apoptosis in a manner that is coordinately initiated but can vary in duration. Proc Natl Acad Sci USA 103:11573–11578.

Nechushtan A, Smith CL, Lamensdorf I, Yoon SH, Youle RJ. 2001. Bax and Bak coalesce into novel mitochondria-associated clusters during apoptosis. J Cell Biol 153:1265–1276.

Newmeyer DD, Farschon DM, Reed JC. 1994. Cell-free apoptosis in Xenopus egg extracts: Inhibition by Bcl-2 and requirement for an organelle fraction enriched in mitochondria. Cell 79:353–364.

Nguyen M, Marcellus RC, Roulston A, Watson M, Serfass L, Murthy Madiraju SR, Goulet D, Viallet J, Belec L, Billot X, Acoca S, Purisima E, Wiegmans A, Cluse L, Johnstone RW, Beauparlant P, Shore GC. 2007. Small molecule obatoclax (GX15-070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. Proc Natl Acad Sci USA 104:19512–19517.

Ni Chonghaile T, Sarosiek KA, Vo TT, Ryan JA, Tammareddi A, Moore Vdel G, Deng J, Anderson KC, Richardson P, Tai YT, Mitsiades CS, Matulonis UA, Drapkin R, Stone R, Deangelo DJ, McConkey DJ, Sallan SE, Silverman L, Hirsch MS, Carrasco DR, Letai A. 2011. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. Science 334: 1129–1133.

Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW, Rosenberg SH. 2005. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 435:677–681.

Pagliari LJ, Kuwana T, Bonzon C, Newmeyer DD, Tu S, Beere HM, Green DR. 2005. The multidomain proapoptotic molecules Bax and Bak are directly activated by heat. Proc Natl Acad Sci USA 102:17975–17980.

Parone PA, James DI, Da Cruz S, Mattenberger Y, Donze O, Barja F, Martinou JC. 2006. Inhibiting the mitochondrial fission machinery does not prevent Bax/Bak-dependent apoptosis. Mol Cell Biol 26:7397–7408.

Pavlov EV, Priault M, Pietkiewicz D, Cheng EH, Antonsson B, Manon S, Korsmeyer SJ, Mannella CA, Kinnally KW. 2001. A novel, high conductance channel of mitochondria linked to apoptosis in mammalian cells and Bax expression in yeast. J Cell Biol 155:725–731.

Ren D, Tu HC, Kim H, Wang GX, Bean GR, Takeuchi O, Jeffers JR, Zambetti GP, Hsieh JJ, Cheng EH. 2010. BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program. Science 330:1390–1393.

Saito M, korsmeyer SJ, Schlesinger PH. 2000. BAX-dependent transport of cytochrome c reconsituted in pure liposomes. Nat Cell Biol 2:553–555.

Schafer B, Quispe J, Choudhary V, Chipuk JE, Ajero TG, Du H, Schneiter R, Kuwana T. 2009. Mitochondrial outer membrane proteins assist Bid in Bax-mediated lipidic pore formation. Mol Biol Cell 20:2276–2285.

Sheridan C, Delivani P, Cullen SP, Martin SJ. 2008. Bax- or Bak-induced mitochondrial fission can be uncoupled from cytochrome C release. Mol Cell 31:570–585.

Suzuki M, Youle RJ, Tjandra N. 2000. Structure of bax. Coregulation Of dimer formation and intracellular localization. Cell 103:645–654.

Terrones O, Antonsson B, Yamaguchi H, Wang HG, Liu J, Lee RM, Herrmann A, Basanez G. 2004. Lipidic pore formation by the concerted action of proapoptotic BAX and tBID. J Biol Chem 279:30081–30091.

Tilley SJ, Saibil HR. 2006. The mechanism of pore formation by bacterial toxins. Curr Opin Struct Biol 16:230–236.

Tilley SJ, Orlova EV, Gilbert RJ, Andrew PW, Saibil HR. 2005. Structural basis of pore formation by the bacterial toxin pneumolysin. Cell 121:247–256.

Tsujimoto Y, Cossman J, Jaffe E, Croce CM. 1985. Involvement of the bcl-2 gene in human follicular lymphoma. Science 228:1440–1443.

van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, Adams JM, Roberts AW, Huang DC. 2006. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 10:389–399.

Van Loo G, Demol H, van Gurp M, Hoorelbeke B, Schotte P, Beyaert R, Zhivotovsky B, Gevaert K, Declercq W, Vandekerckhove J, Vandenabeele P. 2002. A matrix-assisted laser desorption ionization post-source decay (MALDI-PSD) analysis of proteins released from isolated liver mitochondria treated with recombinant truncated Bid. Cell Death Differ 9:301–308.

Vaux DL, Cory S, Adams JM. 1988. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 335: 440–442.

Verhaegen M, Bauer JA, Martin de la Vega C, Wang G, Wolter KG, Brenner JC, Nikolovska-Coleska Z, Bengtson A, Nair R, Elder JT, Van Brocklin M, Carey TE, Bradford CR, Wang S, Soengas MS. 2006. A novel BH3 mimetic reveals a mitogen-activated protein kinase-dependent mechanism of melanoma cell death controlled by p53 and reactive oxygen species. Cancer Res 66:11348–11359.

Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, Vaux DL. 2000. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 102:43–53.

Villunger A, Labi V, Bouillet P, Adams J, Strasser A. 2011. Can the analysis of BH3-only protein knockout mice clarify the issue of 'direct versus indirect' activation of Bax and Bak? Cell Death Differ 18:1545–1546.

Vo TT, Ryan J, Carrasco R, Neuberg D, Rossi DJ, Stone RM, Deangelo DJ, Frattini MG, Letai A. 2012. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. Cell 151: 344–355.

Vogler M, Dinsdale D, Dyer MJ, Cohen GM. 2009a. Bcl-2 inhibitors: Small molecules with a big impact on cancer therapy. Cell Death Differ 16:360–367.

Vogler M, Weber K, Dinsdale D, Schmitz I, Schulze-Osthoff K, Dyer MJ, Cohen GM. 2009b. Different forms of cell death induced by putative BCL2 inhibitors. Cell Death Differ 16:1030–1039.

Wasiak S, Zunino R, McBride HM. 2007. Bax/Bak promote sumoylation of DRP1 and its stable association with mitochondria during apoptotic cell death. J Cell Biol 177:439–450.

Wei MC, Lindsten T, Mootha VK, Weiler S, Gross A, Ashiya M, Thompson CB, Korsmeyer SJ. 2000. tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. Genes Dev 14:2060–2071.

Westermann B. 2010. Mitochondrial fusion and fission in cell life and death. Nat Rev 11:872–884.

Wolter KG, Hsu YT, Smith CL, Nechushtan A, Xi XG, Youle RJ. 1997. Movement of Bax from the cytosol to mitochondria during apoptosis. J Cell Biol 139:1281–1292.

Xu XP, Zhai D, Kim E, Swift M, Reed JC, Volkmann N, Hanein D. 2013. Three-dimensional structure of Bax-mediated pores in membrane bilayers. Cell Death Dis 4:e683.

Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng T-I, Jones DP, Wang X. 1997. Prevention of apoptosis by Bcl-2: Release of cytochrome c from mitochondria blocked. Science 275:1129–1132.

Yang QH, Church-Hajduk R, Ren J, Newton ML, Du C. 2003. Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. Genes Dev 17:1487–1496.

Youle RJ, Strasser A. 2008. The BCL-2 protein family: Opposing activities that mediate cell death. Nat Rev Mol Cell Biol 9:47–59.

Youle RJ, van der Bliek AM. 2012. Mitochondrial fission, fusion, and stress. Science 337:1062–1065.

Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR. 1993. The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 75:641–652.

Zimmermann KC, Ricci JE, Droin NM, Green DR. 2002. The role of ARK in stress-induced apoptosis in Drosophila cells. J Cell Biol 156:1077–1087.

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. 1997. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 90:405–413.