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Review

Bax and Bak Pores: Are We Closing the Circle?

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Bax and its homolog Bak are key regulators of the mitochondrial pathway of apoptosis. On cell stress Bax and Bak accumulate at distinct foci on the mitochondrial surface where they undergo a conformational change, oligomerize, and mediate cytochrome c release, leading to cell death. The molecular mechanisms of Bax and Bak assembly and mitochondrial permeabilization have remained a longstanding question in the field. Recent structural and biophysical studies at several length scales have shed light on key aspects of Bax and Bak function that have shifted how we think this process occurs. These discoveries reveal an unexpected molecular mechanism in which Bax (and likely Bak) dimers assemble into oligomers with an even number of molecules that fully or partially delineate pores of different sizes to permeabilize the mitochondrial outer membrane (MOM) during apoptosis.

Bax and Bak Are Key Players in Mitochondrial Apoptosis

Apoptosis is a form of programmed cell death that occurs in a physiological setting and is indispensable for the preservation of tissue homeostasis and embryonic development. Importantly, apoptosis downregulation is a major cause of tumorigenesis, while upregulation of this process induces infectious, autoimmune, and neurodegenerative diseases [1]. Thus, controlling apoptosis represents an important therapeutic target for the treatment of related diseases [2,3].

The intrinsic or mitochondrial apoptotic pathway, which is common only to vertebrates, is tightly controlled by the Bcl-2 protein family and involves MOM permeabilization (MOMP) [1,4]. Of note, in some invertebrates Bcl-2-like proteins such as CED-9 and EGL-1 of *Caenorhabditis elegans* control cell death, but this process is mitochondria independent. Following MOMP apoptotic factors like cytochrome c and SMAC/DIABLO are released into the cytosol to activate the caspase cascade leading to cell death [5]. Three decades of intense research have led to the identification and characterization of at least 18 different members of the Bcl-2 family and classified them into three subgroups according to their function in apoptosis and the number of Bcl-2 homology (BH) domains they contain. The proapoptotic members of the Bcl-2 family include the multi-BH-domain Bax and Bak, which are considered direct executers of MOMP, and the BH3-only members Bid, Bim, and PUMA, which initiate apoptosis. BH3-only members can be classified as 'direct activators' if they directly bind and activate Bax/Bak or 'sensitizers' if they only bind and neutralize antiapoptotic family members. Finally, the antiapoptotic or prosurvival Bcl-2 proteins Bcl-2 and Bcl-xL contain all four BH domains and inhibit the action of proapoptotic proteins [6]. The functional balance between pro- and antiapoptotic members forms a complex interaction network whose outcome decides cell fate.

Bax and Bak are therefore at the core of the intrinsic pathway of apoptosis [7]. Although both shuttle between the cytosol and the MOM, Bax primarily localizes to the cytoplasm in healthy cells while Bak is mainly located at the MOM. Once activated these proteins oligomerize and

Trends

Interaction with Bcl-2 homology (BH)3-only activators at different sites triggers consecutive conformational changes in Bax that promote its activation at the mitochondrial outer membrane (MOM).

In active Bax the hairpin of helices α5 and α6 adopts a partially open conformation that interacts superficially with the membrane.

Bax assembly at the membrane is initiated by the formation of symmetric dimer units and proceeds by oligomerization into multiple species with heterogeneous stoichiometry.

In the mitochondria of apoptotic cells, Bax clusters into heterogeneous structures including arcs and rings that are associated with membrane pores.

Bax shuttles between the cytosol and MOM in healthy cells and activates independently of BH3-only proteins when prosurvival Bcl-2s are downregulated.

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mediate MOMP, which allows the release of apoptotic factors into the cytosol leading to cell death [4,8].

Here we review the recent progress made in elucidating how Bax and Bak mediate MOMP in apoptosis. We focus on Bax because it has been more extensively studied, but general principles are likely to extend to Bak. Our discussion provides a biophysical perspective of the molecular mechanisms involved and covers different scales of organization: from the conformational changes of Bax and Bak needed for activation in the membrane, through their molecular assembly into oligomers, to the most recent findings about the supramolecular organization of Bax in model systems and cells, which have convinced us that Bax pores exist.

The Deadly Transformation: Assembly Pathway of Bax and Bak

Conformational Changes Drive Bax and Bak Activation

In its inactive, soluble form, Bax has a globular structure comprising nine α helices where a central hydrophobic core (α5) is surrounded by a bundle of amphipathic helices exposed to the aqueous environment [9]. This fold defines a hydrophobic groove, which interacts with the BH3 domain of other Bcl-2 proteins. During apoptosis activation of Bax involves a conformational transition from a globular to an extended, membrane-inserted conformation (Figure 1). An initial structural change allows anchoring of Bax to the MOM through insertion of the transmembrane domain of the C-terminal α9 helix [10,11] while the rest of the protein retains the globular fold. This mechanism seems to be triggered by interaction of cytosolic Bax with the BH3 domain of (activator) BH3-only proteins, which may induce the displacement of α9 from the hydrophobic groove [12] (Figure 1). So far, two activation sites have been identified. One site is located opposite of the canonical hydrophobic groove [11,13] and this 'rear pocket' comprises helices α1 and α6 [13]. Conformational changes following this interaction, rather than direct binding to the hydrophobic groove, would then be responsible for α9 displacement and membrane insertion [11]. Recently, the structure of the interaction site corresponding to the canonical hydrophobic groove has been solved [14,15]. Following binding to direct activator BH3-only proteins, Bax activation proceeds through exposure of the N-terminal segment (which precedes the α1 helix) and rearrangement of the α2/BH3 domain [11,14]. For Bak, further structural details have revealed that movement of α2 initiates dissociation of both the N segment and the α1/BH4 domain from the rest of the protein, with α1 remaining solvent exposed [16].

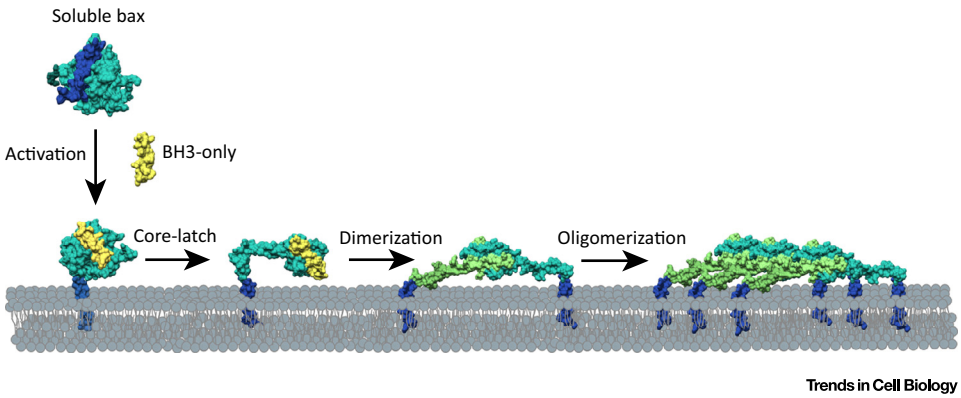

Figure 1. Model for Bax Activation and Oligomerization at the Membrane. Proteins are illustrated in their surface representations. In its soluble form, Bax (cyan) retains a globular conformation where its transmembrane domain (α9 helix, blue) is kept in the Bax hydrophobic groove (α2–α5). On activation by Bcl-2 homology (BH)3-only proteins (yellow), Bax undergoes a series of conformational changes displacing α9 from the groove, which inserts in the membrane (gray), and dissociating in a core (α2–α5) and a latch (α6–α9) domain by unfolding of helices α5 and α6. Following activation, Bax engages in BH3-in-groove dimerization with another activated Bax molecule (light green) while displacing the BH3-only activator from the hydrophobic groove. This leads to the formation of a symmetric dimer. Further oligomerization proceeds by the addition of other dimer units via a second, as-yet unclear, interaction site.

Such conformational changes lead to an opening of the groove that has been suggested to weaken the binding of Bax with its activator partner allowing BH3-only proteins to interact transiently with Bax and 'run away', as proposed in the 'kiss-and-run' model [17]. This step may expose the BH3 domain of Bax, which, free of BH3-only proteins, can then undergo homodimerization. Together these findings are the basis for a model proposing that activation of Bax may follow a two-step process, the first involving α9 transmembrane domain insertion in the MOM and the second requiring BH3 domain exposure for interaction with other Bcl-2 proteins.

Bax and Bak Dimerization and Higher Oligomerization

Crystallographic data for truncated Bax in detergent solution have disclosed a novel conformational change on Bax activation that generates a 'core' (α2–α5) and a 'latch' (α6–α8) domain and induces transient exposure of the α2/BH3 domain [14]. This rearrangement proceeds via partial unfolding of the hairpin comprising helices α5 and α6 [18] and seems to be essential for Bax function [14]. In the dimer the α2/BH3 domain of a Bax molecule binds to the canonical groove of another Bax and vice versa (BH3-in-groove interaction) [14] (Figure 1). This allows the formation of a stable 'symmetric' dimerization domain, which supports a model initially proposed based on crosslinking and low-resolution structural studies [15,19]. This symmetric model was also confirmed using double electron–electron resonance (DEER) studies of the full-length protein in the membrane environment [18] and FRET microscopy in living cells [20]. A similar model has also been suggested for Bak dimerization [21,22].

The existence of an additional interface would provide a rationale for how symmetric Bax and Bak dimers further assemble in higher-order oligomers. Several studies have implicated a role for helices α6 and α9 in Bax oligomerization [15,18,23]. Dimerization via α9 (and probably α6) occurs downstream of BH3-in-groove dimerization, suggesting that it might represent the interaction site of symmetric dimers needed to form higher-order oligomers. Since α9 dimerization is not required for the passage of small molecules like cytochrome c but only for bigger ones like SMAC, the relative importance of interdimer contacts via helices α6 versus α9 remains unclear. Recently, crosslinking and DEER results have reported a novel oligomerization site for Bak in isolated apoptotic mitochondria involving the α3/α5 interface, which, together with α6 (and hypothetically α9), stabilizes interactions of neighboring Bak homodimers [24].

Furthermore, the structural arrangement through which Bax oligomers perforate the MOM remains unknown. In the longstanding 'umbrella' model, inferred on the grounds of structural similarities between soluble Bax and other α-pore-forming proteins like colicin and diphtheria toxin [25], the α5 and α6 helices were assumed to insert as a transmembrane hairpin perpendicularly to the membrane while the other helices would lie on the membrane surface in an umbrella-like configuration. However, this model does not seem to hold true in light of recent data on active Bax and Bak in membranes. During BH3-in-groove dimerization, the α4 and α5 helices generate an aromatic planar surface that may sit on the membrane [14]. Insertion of hydrophobic aromatic residues into the lipid bilayer might increase membrane tension and favor a positive membrane curvature that would engage MOMP [8,14,26]. The result is an 'in-plane' model where insertion into the membrane occurs completely for the α9 but only shallowly for the α5 and α6 helices [14,26]. How Bax and Bak domains are arranged with respect to such a membrane pore is unclear in this model. Independent studies suggest that intersected or parallel α9 helices are located at the rim of the pore [23].

DEER studies of membrane-bound full-length Bax provided additional information about the structure of active Bax dimers in the membrane [18]. While the α2–α5 core helices of Bax dimers retain a well-defined structure similar to the soluble one, helices α6–α9 adopt a more flexible conformation, which may play a role in membrane destabilization. This is the basis for a model where partial opening of helices α5 and α6 is essential for membrane permeabilization;

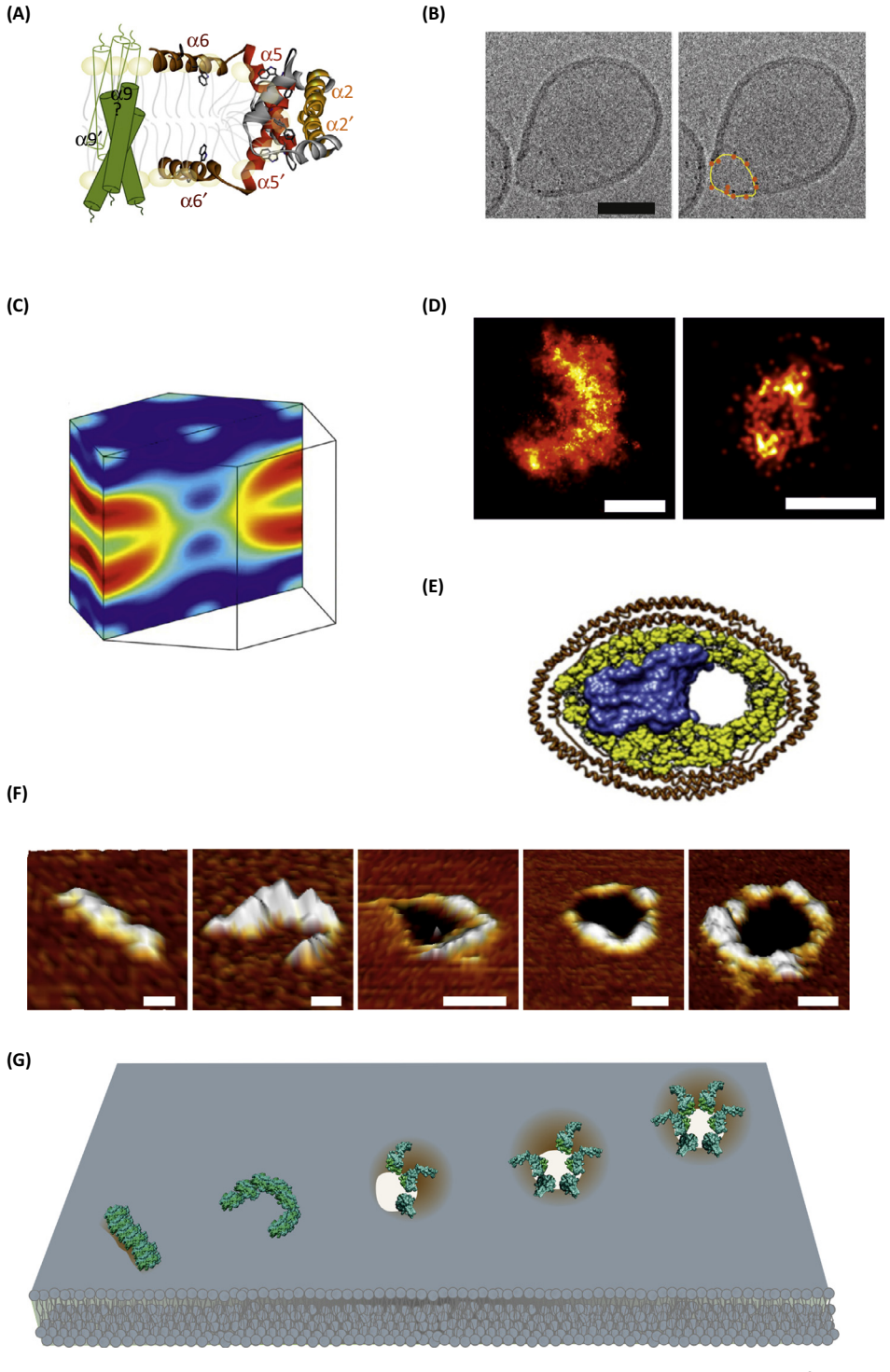

Figure 2. Evidence of Bax Pore Formation. (A) Clamp model of the Bax dimer at the membrane. The dimerization domain is at the edge of a membrane pore induced by Bax while α6 helices are located on opposite sides of the membrane. The orientation of the C-terminal α9 helices is unclear. Reproduced, with permission, from [18]. (B) Cryoelectron microscopy (cryo-EM) image of gold-labeled Bax delineating a pore in the membrane of a vesicle. In the corresponding image, the pore is outlined in yellow and Bax in orange for visual purposes. Bar, 40 nm. Reproduced, with permission, from [51]. (C) X-ray diffraction structure of a pore formed by the α5 peptide of Bax. Yellow-to-red regions represent high electron

(Figure legend continued on the bottom of the next page.)

these two pairs of helices assemble in the dimer as a 'clamp', with the dimerization domain (helices α2–α5) at the rim of a lipidic pore and the amphipathic α6 helices lying on the surface, parallel to each other, on opposite sides of the membrane [18] (Figure 2A). In this model it is unclear how the C-terminal α9 helices are oriented. They could be antiparallel, unless the structural symmetry of the dimer is broken. Despite this, if confirmed, this model would provide a firm explanation for how Bax molecules stabilize open pores at the membrane.

Insight into the Assembly of Bax Oligomers

Deep structural changes induce a conformation of Bax that inserts extensively into the lipid bilayer and perforates it [2]. This step is concomitant with Bax assembly into oligomers. Crosslinking and gel filtration studies have detected diverse oligomeric forms of Bax [27,28]. However, none of these experimental approaches allowed precise determination of the oligomeric state of Bax during its activation and function in the membrane.

Single-molecule imaging studies have recently elucidated key aspects of the molecular mechanism of Bax assembly in the membrane [29]. Initially, Bax inserts into the bilayer as a monomer, suggesting that Bax activation involves conformational changes that allow its membrane insertion but precede its dimerization, supporting a two-step activation process for Bax. However, probably due to the instability of this monomeric structure, Bax molecules quickly self-assemble into higher-order oligomers. Importantly, in equilibrium, Bax in the membrane is present as a mixture of species based on dimer units [29] (Figure 1). The idea of a dimerization unit is attractive. On the one hand, it is in agreement with the structural data on active Bax in membranes [14,18]. On the other hand, it implies that interdimer interactions are weaker than intradimer ones, in line with a dynamic vision of Bax assembly where oligomerization is a reversible process that can be modulated, for example, by protein density at the membrane. This would explain the variety of oligomeric species found in previous studies and would be consistent with the formation of membrane pores that are tunable in size [30,31].

These data also suggest that, although BH3-only proteins are important for Bax activation, they do not affect the final distribution of oligomeric species. It remains unclear, however, whether the BH3-only proteins bind only to Bax monomers or also to oligomers and, in that case, whether they are released as the oligomers grow. Interestingly, Bcl-xL was able to disassemble preformed complexes. Although the exact molecular mechanism is unclear, this suggests that the assembly pathway of Bax is a reversible process that can be regulated in both directions by several factors. In this context it has been reported that mitochondrial size influences Bax activity, suggesting a role for membrane curvature in Bax oligomerization [32]. Additional open questions include how, given a finite amount of Bax molecules available, the number and size of Bax oligomers/pores is controlled during apoptosis and how other Bax-interacting proteins, like Bak or VDAC2, affect these oligomers.

Bax, Bak, and the Pore

At a larger length scale, the nature of supramolecular structures formed by Bax and Bak to mediate MOMP has remained one of the key questions in the field. Several studies on model membranes have characterized Bax and Bak as pore-forming proteins capable of disrupting the

density of Br atoms localized at the lipid chains. Electron density is low (blue regions) at the pore rim and outside the membrane. Reproduced, with permission, from [45]. (D) Arc and ring structures of GFP-Bax wild type reconstructed by super-resolution single-molecule localization microscopy. Bars, 100 nm. Reproduced, with permission, from [48]. (E) Model of nanodisk structure (yellow) containing a single unit of BAX/BID peptide (blue surface) based on cryo-EM data [50]. Reproduced, with permission, from [82]. (F) Atomic force microscopy (AFM) images of structures made by Bax (yellow protrusions) on supported lipid bilayers (brown areas) showing (left to right) a line, arcs of different lengths, and a ring. The black area in the last three images is representative of a hole in the membrane. Bax molecules partially or completely line the pore rim. Bars, 50 nm. (G) Models for Bax (cyan) structures, including (left to right) lines, arcs, and rings, leading to pore formation in the membrane (gray).

continuity of the lipid bilayer by opening toroidal pores. Toroidal pores are pore structures where both proteins and lipids form part of the pore wall [33]. As a result, not only Bax and Bak but also membrane mechanical properties play a role in the size and stability of these pores [34].

It is important to consider that membranes are noncovalent 2D assemblies kept together by the hydrophobic effect. This means that disrupting the continuity of the membrane bilayer has a high energetic cost. Therefore, it is not trivial how a protein would induce membrane opening. When membranes are under stress they are tensed, and beyond a certain threshold the planar assembly disrupts and a pore opens [35]. The toroidal pore model proposes that lipids at such a pore rim bend to avoid exposure of the hydrophobic acyl chains to the aqueous environment [33]. As a consequence the two membrane leaflets form a continuous surface that is brought into contact by lipids at the curved pore edge. It is this high membrane curvature that is responsible for the high energy cost of maintaining the open pore conformation. The energy cost is directly proportional to the perimeter or length of the pore. For this reason the driving force for toroidal pore closure is known as line tension (in contrast to membrane or surface tension, which is proportional to the area). To keep a toroidal pore stably open, the energy cost of the bent membrane at the pore edge needs to be reduced by stabilizing the curved structure [34]. In such a scenario, Bax and Bak would need to first stress the membrane – for example, by asymmetric binding to the cytosolic leaflet of the MOM and by high local protein concentration associated with oligomerization, thereby inducing pore opening – and then stabilize the pore by decreasing the line tension. In reconstituted systems Bax alone is able to stress the membrane, promote pore opening, and stabilize long-lived pores [36,37]. However, it is important to note that other components present at Bax foci might contribute in live cells.

There is accumulating evidence in favor of the toroidal pore model for Bax and Bak. Classical experiments involved the Bax-dependent decrease in the lifetime of planar bilayers [38], the formation of large membrane openings modulated by lipids with intrinsic curvature [39,40], and the movement of lipids between the two leaflets in Bax-permeabilized membranes [41]. Cryoelectron microscopy images of liposomes revealed round membrane holes of 25–100 nm in diameter in the presence of active Bax (or Bak) [19,42,43] (Figure 2B). These membrane pores were long lived and tunable in size, depending on the density of Bax/Bak molecules on the membrane surface, and were associated with the formation of Bax and Bak oligomers [30,31,36,44]. The most convincing evidence so far for continuous lipid monolayer organization in a toroidal pore was obtained with a peptide derived from helix α5 of Bax [45] (Figure 2C) that reproduced the pore-forming properties of the full-length protein [46,47]. However, the formation of such pores in apoptosis, as well as the distribution of Bax and Bak molecules with respect to the pore rim, remained obscure.

Recently, the formation of Bax assemblies in the form of arcs and rings associated with membrane pores at the MOM of apoptotic cells has been observed [48,49] (Figure 2D). In agreement with experiments on model membranes, Bax oligomers displayed a wide distribution of sizes (ranging from a few tens of nanometers to 100 nm in diameter) and shapes, indicating that Bax-mediated MOMP involves flexible and diverse pore structures. Additional atomic force microscopy (AFM) experiments on supported bilayers and electron microscopy of outer membrane vesicles (OMVs) and lipid nanodisks containing Bax confirmed protein enrichment at the rim of membrane pores of variable sizes where the pore wall was not completely covered by protein molecules [48,50,51] (Figure 2E,F). Similar arc structures that only partially cover the pore ring have recently been reported for other pore-forming proteins [52–55]. Quantitative analysis of Bak clusters *in situ* by single-molecule localization (SML) microscopy have shown a broad distribution of Bak cluster sizes in apoptotic mitochondria, although protein density remained homogeneous among clusters [56]. Unfortunately, no defined structures could be identified [56]. Together, the evidence reveals an unexpected model for Bax- (and probably Bak-) mediated

MOMP where oligomerization at the MOM induces the formation of heterogeneous toroidal pore structures that are flexible and evolve over time (Figure 2G) leading to the release of apoptotic factors.

The question about the minimum number of Bax molecules able to form a functional pore at the MOM remains open. Data from lipid nanodisks suggest that monomers or dimers are sufficient to stabilize membrane openings [50], which could grow with additional Bax molecules. Another issue is the contribution of other MOM components to pore structures, including Bak [57] and proteins involved in mitochondrial dynamics such as Drp1 and Mitofusins [58]. The similar ability of Bak to form membrane pores [31,42,59] raises the question of whether they cooperate to form mixed assemblies at the MOM and whether these assemblies include mixtures of homodimers or heterodimers of Bax and Bak, as previously suggested [15,60]. Recent data suggest that Bok, a poorly understood member of the Bcl-2 family, is able to induce MOMP in the absence of Bax and Bak and to form pores in liposomes [61,62]. Further research is needed to understand whether Bak and Bok induce pore assemblies comparable with Bax and to shed light on the structural and functional consequences of potential cooperation between these proteins.

Regulation of Bax and Bak Activity

A longstanding debate in the apoptosis field revolves around the regulation of Bax (and Bak) activity. The 'direct activation' model [40], as well as the 'embedded together' and 'unified' models [63,64], assumes that Bax requires activation by a 'direct activator' BH3-only protein to mediate MOMP. Antiapoptotic Bcl-2 proteins play their inhibitory role by binding either directly to the executioners Bax and Bak or to BH3-only proteins, which then are no longer available to activate Bak/Bax [63]. The counterargument is provided by the 'neutralization' model [65], which proposes that antiapoptotic Bcl-2 proteins sequester constitutively active Bax/Bak in healthy cells and inhibit MOMP until apoptotic stimuli induce BH3-only proteins to displace them from the inhibitory complexes (Figure 3).

Biochemically, Bax can be produced as an inactive, soluble monomer in the absence of detergents and become efficiently activated by the addition of substoichiometric amounts of

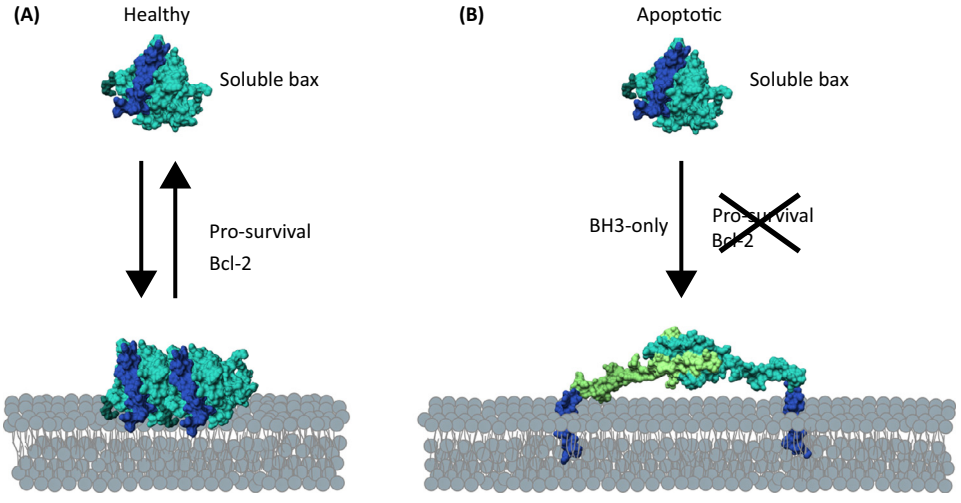

Figure 3. Regulation of Bax Activity by Other Bcl-2 Proteins. (A) Under healthy conditions Bax (cyan) associates with the mitochondrial outer membrane (MOM) (gray) but is continuously retrotranslocated to the cytosol by prosurvival Bcl-2 proteins. (B) During apoptosis Bcl-2 homology (BH)3-only proteins break this steady state by inhibiting the action of prosurvival Bcl-2 proteins and activating Bax. Insertion of α9 (blue) in the membrane and further conformational changes allow Bax oligomerization and induce MOM permeabilization (MOMP).

activator BH3-only proteins or derived peptides [40,66,67]. Moreover, several structural studies have revealed sites of direct interaction between Bax/Bak and BH3-only proteins or peptides, demonstrating a role for these proteins in catalyzing the activity of Bax [13,14,68]. However, *in vitro* Bax activation can also be achieved by other factors, like acidic pH [69], detergents [19,70], oxidized lipids [71], or mild heat treatment [72], provided that a lipid bilayer is available. Even membrane-bound Bax can autoactivate inactive Bax molecules [73]. Together these findings favor a low energy barrier for Bax transition from the soluble to the membrane-inserted conformation. Several genetic studies that knocked out increasing numbers of BH3-only proteins [74,75] revealed that the resulting cells differed from the double Bax/Bak knockout phenotype despite also becoming increasingly resistant to apoptotic stresses [76]. It was contemplated that autoactivation of Bax and Bak via downregulation of Bcl-2, Bxl-xL, and Mcl-1 was occurring in the absence of Bid, Bim, Puma, and Noxa [75]. Moreover, systematically knocking out all established members of the Bcl-2 family [77] and reintroducing Bax alone led to MOMP even at low expression levels. Overall, these findings add new support to the neutralization model, although the existence of unknown factors capable of directly activating Bax cannot be excluded and the clear experimental corroboration of the direct activation model suggests that the main pathway for the regulation of Bax/Bak activity may depend on the *in vivo* setting.

The picture has become more complicated by the realization that Bax (and to a lesser extent Bak) continuously shuttles between the cytosol and mitochondria of healthy cells independent of BH3-only proteins [78–80]. This retrotranslocation process is regulated by prosurvival Bcl-2 homologs, which bring Bax back to solution and maintain nontoxic, steady-state levels of Bax at the MOM [81]. Although the extent of Bax insertion in the lipid environment of the MOM that remains competent for retrotranslocation is not well defined, this process requires the removal of protein–lipid interactions, which theoretically should be thermodynamically unfavorable. Unless a molecular mechanism that provides energy for retrotranslocation is discovered, one could also reason that the dissociation of Bax from the membrane could proceed spontaneously via a low energy barrier accessible by thermal fluctuations. This idea would reconcile the diverse models for the regulation of Bax activity, although additional research is needed to validate it.

In summary, it is likely that both constitutive and direct activation of Bax and Bak are regulated by other Bcl-2 members. It is likely that aspects such as cell type, metabolic conditions, expression levels of different Bcl-2 members, or the death-priming state of the cell play a role in the balance between soluble and MOM-associated Bax and in the required conditions (prosurvival Bcl-2 neutralization and/or direct activation by BH3-only proteins) for switching Bax to pore formation and MOMP.

Concluding Remarks

The past years have been crucial in elucidating the mechanisms involved in Bax and Bak activation and organization in oligomeric structures that mediate MOMP. A much clearer picture is now being defined where, on activation, Bax undergoes a series of conformational changes that lead to the formation of dimers that further assemble into higher oligomers. Both the stoichiometry and the nanoassembly of these oligomers are diverse, but they promote the formation of heterogeneous pores at the MOM that can be partially or fully lined by Bax molecules. These recent discoveries have questioned previous findings and have prompted new and challenging questions (see Outstanding Questions) such as the minimal number of Bax molecules able to open a pore, the structural disposition of Bax on these pores, and the participation of additional molecules in the structure. New experimental approaches combining multiple types of information (e.g., pore functionality with protein structure) will contribute to answering these questions.

Outstanding Questions

What is the nature of the interaction surfaces that mediate interdimer interactions and the formation of higher-order Bax oligomers?

What is the structural arrangement of active Bax dimers with respect to the membrane pore? How do the flexible regions in active Bax contribute to its function?

What is the stoichiometry of Bax in complex with BH3-only proteins and prosurvival Bcl-2 and how do these interactions modulate Bax oligomerization?

What is the minimal oligomeric form of Bax capable of stabilizing membrane pores? How does Bax oligomerization correlate with pore size? Is there a maximum or preferred size for Bax oligomers?

How do Bax oligomers interplay with additional proteins in the context of the pore? Do Bak and Bok form apoptotic pores similar to Bax?

What is the relative importance of Bax spontaneous activation versus direct activation by BH3-only proteins to promote apoptosis? What is the mechanism behind the switch in Bax steady retrotranslocation and accumulation and activation at the MOM?

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