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Review

The Bax pore in liposomes, Biophysics

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Abstract

The protein BAX of the Bcl-2-family is felt to be one of the two Bcl-2-family proteins that directly participate in the mitochondrial cytochrome *c*-translocating pore. We have studied the kinetics, stoichiometry and size of the pore formed by BAX in planar lipid bilayers and synthetic liposomes. Our data indicate that a cytochrome *c*-competent pore can be formed by in-membrane association of BAX monomers.
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Keywords: apoptosis; Bax; cytochrome *c*; pore

Abbreviations: TNF, tumor necrosis factor; CF, carboxyfluorescein; FITC, fluorescein isothiocyanate

Introduction

Genetically programmed cell death occurs in all multicellular organisms.[1–3] The function of Bcl-2-family of proteins is to govern the central decision point within the mitochondrial (intrinsic) apoptotic pathway.[4–10] This protein family includes both proapoptotic (e.g. Bax, Bak, Bcl-x_s, Bad, Bik, Bid, Hrk, Bim, Noxa) and antiapoptotic (e.g. Bcl-2, Bcl-x_{LL}, Mcl-1, A1, Bcl-w) molecules.[11–15] Members of these two classes neutralize each other's effect and therefore heterotypic combination is critical for controlling apoptosis.[16–18] In addition, homotypic combination to higher order oligomers has been shown to form the large cytochrome *c*-transporting pores.[19–23] It is now universally accepted that oligomerization is decisive in the mitochondrial death decision, but the composition of the critical regulatory oligomers remains to be resolved. The biochemical activity of these proteins was revealed by the first structure of Bcl-x_L, which showed a remarkable protein fold.[24] The structure of this antiapoptotic protein resembled closely the fold of the pore-forming subunits of the colicin/diphtheria toxin molecules (Figure 1). This observation correctly focused interest on the membrane association and the in-membrane pore-forming activities of the Bcl-2-family proteins.[25–28] The realization that cytochrome *c* release from the mitochondria was the essential consequence of Bcl-2-family protein activity[29–32] has crystallized our focus on oligomerization and pore formation by this protein family. Stan Korsmeyer and his collaborators were instrumental in defining the Fas (TNF)–Bid–Bax/Bak pathway of apoptosis regulation.[4,6] However, as many researchers have observed, the Bcl-2-family apoptosis decision pathway is complex, interdigitates at multiple levels with other cellular pathways and employs biochemically diverse mechanisms. The biochemical characterization of 'active' Bcl-2-family proteins and the control of such activities will be central to understanding the control of apoptosis. We appreciate that the death decision requires translocation of Bcl-2-family proteins to the outer mitochondrial membrane, oligomerization of trans-located and resident proteins in that membrane and the activation of pores to release cytochrome *c* as well as other mitochondrial proteins into the cytoplasm. However, this summary of the mitochondrial death decision is not complete. Our investigations have attempted to incorporate additional details about the mitochondrial death decision by using well-defined experimental systems that permit quantitative analysis.

Some Structural/Functional Homologies Among Bcl-2-Family Proteins

The solution and crystal structures of Bcl-x_L, Bcl-2, Bid and Bax demonstrate a similar three-dimensional arrangement of the helical segments (Figure 1).[24,33–36] The Bax and Bid structures are representative of the cytoplasmic 'inactive' forms of the respective proteins. The Bcl-2 and Bcl-x_L structures are not easily associated with a functional state of the respective proteins and the membrane structures of all of these proteins remains to be determined. The proteins in Figure 1 have 7–9 α-helices arranged in three layers. The colors used are intended to suggest regions of similar function in the different proteins. In some Bcl-2-family proteins, the green amphipathic helices are proposed to contribute to channel formation by traversing the membrane.[24,27,37–39] This hairpin is proposed to extend, thereby forming the handle of an umbrella with the remaining helices distributed on the insertion surface of the membrane.[40,41] Although these models only suggest events in membrane insertion, they have been used to develop the concept of activation of the soluble proapoptotic Bcl-2-family members.

Bid activation in TNF signaling

In the case of Bid, the N-terminal 'insertion-blocking' helices pack against the hydrophobic core and are proteolytically removed for activation.[6,35,36] The cleavage of bid results from TNF receptor ligation and subsequent activation of caspase-8.[42,43] This cleavage, when followed by posttranslational myristoylation, makes bid very active at translocation to the mitochondria.[44] It seems clear that the activation of bid occurs

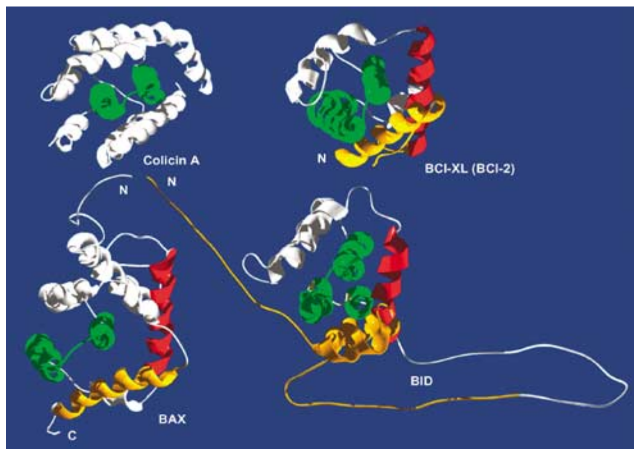

Figure 1 Bcl-2-family structures with colicin. These models were assembled from the deposited PDB files for Bax, Bcl-x_L, Bid and Bcl-2, with Bcl-x_L.[24,33–36] The models were colored as follows: green – hydrophobic core helices; red-helix containing BH3;, gold-'insertion-blocking' helix associated with the hydrophobic core. The structures are rotated so that these domains appear similarly in each panel

by exposing the hydrophobic helices and approximating them to the target membrane. The detailed series of events from ligation of TNF to the translocation of activated bid to the mitochondria was developed extensively in Stan Korsmeyer's laboratory and confirmed the paradigm of the mitochondria death decision as central in Bcl-2—family-mediated apoptosis.[45,46]

Bax activation for membrane insertion and pore formation

All of the Bcl-2-family structures shown in Figure 1 have possible 'insertion-blocking' helices, indicated by gold coloration. For membrane resident Bcl-x_L and Bcl-2, the function of these helices is not clear. For the soluble protein, Bax, the solution structure represents the cytosolic resident and physiologically inactive form. Stan Korsmeyer suggested that since the TM region of Bax was not required for proapoptotic activity in mammalian cells[47] and partial removal of the hydrophobic TM region, Bax(ΔC19), reduced aggregation of purified preparations,[48] this might be useful for biophysical studies. We found that the Bax(ΔC19) after a urea denaturation/renaturation cycle was monodisperse by light scattering[49] and was more active than full-length that was activated with detergent, Figure 3a. This engineered preparation, Bax(ΔC19), was used to study the biophysics of cytochrome *c*-competent pore activation in define liposomes and planar lipid bilayers.[25,49]

Structural Requirements for Pore Formation

Once the death signal has been transmitted to the mitochondria, the release of cytochrome *c* and other proteins must be accomplished to complete the death decision. After translocation to the mitochondria, the activated Bid will not itself release cytochrome *c*. The release of cytochrome *c* requires the oligomerization of Bak or Bax in the outer mitochondrial membrane.[6,50] In Figure 1, the red helices include the BH3 region that associates with the hydrophobic core in all Bcl-2-family proteins, thus far identified. Initially, this region was implicated in specific recognition between soluble dimers[51,52] and is now known to be a major contributor to the in-membrane oligomerization and pore formation.[19,53] The structure of the stable cytosolic Bax recapitulates the theme of amphipathic α-helices forming an envelope around the core hairpin helix. In this case, the C-terminal helix 9 partially covers the hydrophobic core. *In vitro* and *in vivo* activation studies indicate that mitochondrial membrane insertion occurs in parallel with displacement of helix 9 from this position.[34,54–56] We determined that by shortening this helix we could generate an active form, Bax(ΔC19), for biophysical studies in liposomes and planar lipid bilayers.[25,49] Extensive mutation studies confirm that the Bax BH3 helix mediates binding between Bcl-2-family proteins.[19,51,57–59] Although central to the regulation of apoptosis, a single binding site will provide only for specific dimerization. The higher order oligomers important in mitochondrial dysfunction and protein release require at least two sites of interaction per Bcl-2-family member.[60,61] The structural dependence of the oligomerization remains to be determined. It seems likely that the homo-oligomerization of Bax and Bak, which is required for cytochrome *c* release, is an excellent candidate for a domain swapping mechanism.[62,63]

Channels Formed by Bcl-2-Family Proteins

Initially, four papers were published in which Bcl-x_L, Bcl-2 and Bax were demonstrated to display channel-like activity in planar lipid bilayer experimental systems.[25–28] In all cases, pore formation was enhanced by acid pH. As the pIs of these proteins vary from 7 to 4.5, it seemed unlikely that this pH dependence was reflecting a common mode of activating the individual proteins. The pores formed by these purified proteins did have some common properties. In each case, multiple conductance levels were identified for each protein that was reconstituted into planar lipid bilayers. This suggested that the individual proteins formed multiple homo-oligomeric structures in the membrane that generated the pore activity. The first channels to appear were always small and produced the simplest current recordings. Subsequently, larger currents and more complex patterns appeared, indicating that sequential oligomerization was increasing pore size. We determined by light scattering that our Bax preparation was monomeric. These characteristics suggested to us that the Bcl-2-family proteins entered the membrane as a monomer and undertook multimerization in the membrane. During this association, the oligomers activate to ion-conducting channels of various sizes. The resulting channels demonstrated low ion selectivity that seemed to primarily result from electrostatic properties of the proteins forming the pore. For example, Bax and Bcl-x_L were proteins with a low pI and displayed a preference for anions. However, Bcl-2 with a higher pI was slightly cation selective. We found no selectivity

requiring a highly developed ion-selectivity filter in the Bcl-2-family channels that were formed.

We observed the soluble protein preparations incorporated into liposomes and planar lipid bilayers in a pH-dependent manner. At low pH, the rate of incorporation was rapid at nanomolar protein concentrations and generated membrane pores and channels. The difference in membrane incorporation was small in spite of the large difference in the protein pI: Bcl-2 (7.12) and Bax (4.96). We therefore concluded that pH dependence was owing to local charge neutralization on portions of the Bax and Bcl-2 molecules. However, in both cases, the pH-dependent insertion of Bax and Bcl-2 was into a lipid composition that included negatively charged lipids. Therefore, pH-dependent reduction of the membrane surface charge was contributing to the rapid membrane incorporation at pH = 4. After renaturation from 6 M urea, our Bax protein was consistently active between 1 and 50 nM protein concentration. Over this range, it was possible to show that the Bax channel activity was concentration dependent. In Figure 2, the pattern of Bax currents at 5 and 20 nM added protein is shown. At the higher concentrations only the larger, ~11 pA channel is observed. Under these conditions, this channel has open times as long as 500 ms. At 5 nM, the large channel flickers open and with the limited frequency response of the planar lipid bilayer the amplitude of this channel was probably underestimated. Using a simple model of two open and one closed state for this channel, it was possible to estimate the kinetic constants for these transitions and to successfully simulate both the low- and high-concentration current patterns.[64] Assuming that the current states represented the Bax monomer, dimer and tetramer, respectively, the formation constants for dimerization and tetramer formation are $\sim 4 \times 10^7\,M^{-1}$ Our bilayer studies indicated that the channel activity of Bax displayed a concentration dependence that reflect in-membrane oligomerization. With time we observed the Bax and Bcl-2 channels to grow to nano-Siemen size in planar lipid bilayers.[25] This was a membrane lifetime that could not be reproduced in the liposome experiments. This activity is like that observed by others in mitochondria[65–68] and may represent another level of organization, which we have not been able to study.

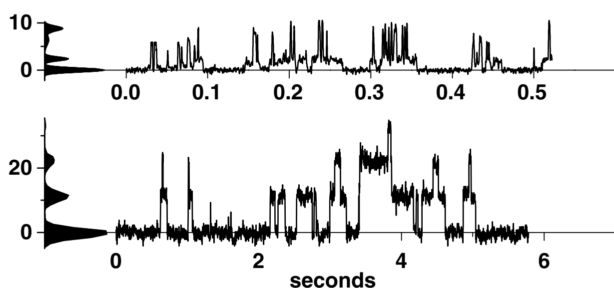

Figure 2 Concentration dependence of bax channels. Genetically engineered and renatured Bax(ΔC19) was incorporated into asolection bilayer membranes at pH 4. Subsequently, the pH was increased to 7. The upper trace was acquired at a Bax concentration of 5 nM and the lower trace was obtained at 20 nM bax. All points histogram analysis is shown on the *y*-axis. Current levels were 1.97, 6.05 and 8.81 at 5 nM and at 20 nM one current transition of 11.3 ± 0.17 that is repeated

The Biophysical Properties of the Bax Pore

Using a mono-disperse preparation of liposomes, 182 ± 12 nm, we undertook the quantitative study of Bax pore formation. These liposomes contain carboxyfluorescein (CF) at quenching concentrations, so that the dequenching resulting by release from the liposome interior produced a very sensitive assay of pore formation.[69] The rapid diffusion of CF from vesicles of this size meant that we could consider that each pore formed would empty its vesicle and that the kinetics of pore activation could be monitored experimentally by the dequenching rate.[70,71] The time course of dequenching is described by:

$$R_t = A_1(1 - e^{-(t/\tau)}) + mt \quad (1)$$

where R_t is the fractional release at time, *t*. Because of the uniform vesicle size, 182 ± 12 nm, the unilamellar vesicles empty in ~30 ms and the kinetics of dequenching represent pore activation. The rapid exponential component dominates dequenching and is characterized by a time constant, τ, and the exponential fraction, A_1. A minor linear component was also observed under some conditions and included in the fitting equation. The exponential dequenching has been studied in detail and used to characterize the stoichiometry of pore activation (see below). The linear release can be attributed to rare circumstance when the pore open time is shorter than the vesicle emptying time, $\tau_{pore}/\tau_{empty} < 1$.[70,72] Using this experimental system, we proceeded to a quantitative analysis of pore stoichiometry and pore size.

The data shown in Figure 3b are plotted according to the Hill equation,[73] with the Bax concentration corrected for the lipid concentration present as liposomes. This represents an initial attempt to estimate the effect of lipid amount on the pore formation. This effect was first noted when studying the Bax-mediated release of FITC-cytochrome *c* from liposomes, where higher concentrations of liposomes were required to overcome the decreased sensitivity of the FITC-dequenching. In this instance, we needed to go to high Bax concentration in order to generate FITC-cytochrome *c* release. When corrected for lipid concentration, the release of CF and FITC-cytochrome *c* occurs at the same Bax densities in the membrane, Figure 3b. This plot makes it clear that the stoichiometry of pore activation governs the molecules, which can exit through the pore. The Hill plot of CF release is a composite of two linear curves having slopes of 2 and 4. This is consistent with the dimer–tetramer transformation that is observed in the bilayer concentration dependence. When FITC-cytochrome *c* is reporting the pore activation, only the tetramer pore is detected, resulting in a linear Hill plot with a slope of 4.

The clear implication of Figure 3b is that the tetramer pore is larger than the dimer pore. To study this directly, we employed dextrans of molecular weight from 1.5 to 66 kDa to block either the CF or FITC-cytochrome *c* dequenching. When CF was the dequenching probe, the dimer pore has a diameter of 11 Å and the tetramer pore a diameter of 22 Å. When pore size was estimated by blocking the dequenching of FITC-cytochrome *c* with dextran, the tetramer pore was determined to have a diameter of 28 Å. We concluded that this increased size

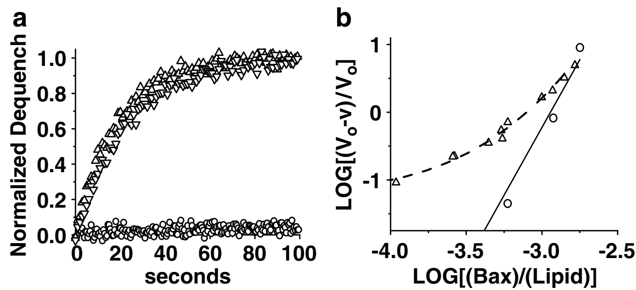

Figure 3 Kinetic analysis of Bax pores in liposomes. Liposomes of uniform size and lamellarity were prepared as described in Saito *et al.*[49] Using CF and FITC-cytochrome *c* dequenching, the concentration dependence and stoichiometry of Bax pore activation were studied in detail. (**a**) A comparison of the time dependence of pore activation in liposomes for full-length mouse Bax (O, 180 nM), detergent-activated full-length mouse Bax (△, 120 nM) and Bax(ΔC19) (−, 9 nM). The plots are normalized to the total liposome dequenching resulting from 1% Triton X-100. From the time constants of the exponential activation of Bax pores, τ, we have studied the stoichiometry of pore activation. (**b**) Using the time constants of pore activation, we have employed Hill analysis and plots of that data to study the stoichiometry of pore activation. Dequenching of CF can be fitted by a curve (dashed line) that is the sum of two linear dependencies of slopes 2 and 4. This contrasts with the Hill plot of the (BAX) dependence of FITC-cytochrome *c* dequenching (−), which can be fitted to a linear dependence of slope 4. These data have been normalized for vesicle concentration so that the *x*-axis is the molar fraction of added protein to added lipid. This permits a direct visual comparison of the concentration dependence of CF and FCC release. From the original data, we determined that the dimer pore represented 92% of the added Bax(ΔC19) at 5 nM and the tetramer pore $>$88% at 20 nM. The renatured Bax(ΔC19) was monomeric in solution by light scattering and these data represent the stoichiometry of 'in-membrane' homotypic associations

reflected a direct interaction between cytochrome *c* and the Bax tetramer during pore transit.

From these experiments, we formed some conclusions about the nature of Bax pores. Bax appears to enter the membrane as a monomer where the membrane environment enhances its ability to form oligomers. The resulting oligomers of Bax form membrane pores of two sizes. This oligomerization is driven by the concentration of Bax in the membrane. Bax alone is sufficient to form a pore, which can transport cytochrome *c* across the bilayer. During cytochrome *c* transit, there is a direct interaction with Bax that is reflected in an increase in pore size.[49] These conclusions lead to the prediction that the membrane environment, including lipid composition and membrane proteins, can have a direct effect upon Bax pore activation and size. The cardiolipin of mitochondria is reported to enhance the large pore formation by Bax.[74] In the mitochondria tBid, Bcl-x_L, Bcl-2 and VDAC have all been observed to affect Bax pore activity.[6,75,76] In addition, detergent micelles can be used to activate the full-length Bax.[34,61,77] Furthermore, it has recently been suggested that Bax and Bak form homotypic oligomers in the mitochondrial outer membrane.[21,22] In summary, our study of the pore activation in liposomes appears to have provided an accurate model of part of the mitochondrial death decision and even produced a limited predictive model for in-membrane and lipid effects.

Looking at a Bax pore, indirectly

We have studied the biophysical properties of a single Bax channel in planar lipid bilayers. Using dequenching from

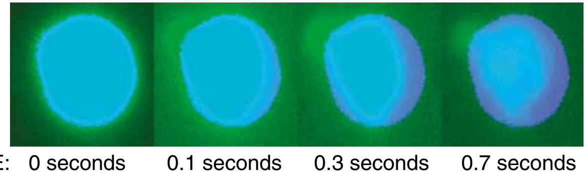

Figure 4 Giant vesicles with a Bax pore. Giant liposomes were prepared using Bax 30 mol% DOPA and 70 mol% DOPA following a published procedure.[78] The liposomes were purified and concentrated by centrifugation immobilized on glass coverslips with 100 μM polylysine, as described. Bax(ΔC19) ($\sim$10 nM) was added to the buffer suspending the liposomes and a time series of images was collected. The images displayed here show the localized release of fluorescence from giant liposome and the elapsed time of the release. Liposomes were allowed to tether to the coverslip surface before measurements were begun

fluorescein-loaded liposomes, the macroscopic behavior of Bax pore activation and cytochrome *c* transportation has been quantitatively explored. However, it remains to integrate how the single Bax pore properties are reflected in the macroscopic cytochrome *c*-transporting function.

In an attempt to implement this integration, we have employed the release of CF from giant liposomes. Small, for example, 200 nm, liposomes are emptied within 30 ms after Bax pore formation. Because it was not possible to study the transport kinetics of CF, cytochrome *c* and dextran from 200 nm liposomes our previous work focused on pore activation. The giant liposomes were prepared with the same lipid composition, 30 mol% DOPA and 70 mol% DOPC, used in the studies of pore activation and following a published procedure.[78] With the CF added at nonquenching concentrations, it is possible to follow it exit through a Bax pore, as shown in Figure 4. The large size of the liposome extends the release of CF over $\sim$1 min. The decrease of intravesicular fluorescence and the point of CF exit from the liposome were both observable by fluorescence microscopy. We have successfully captured the formation of single Bax pore complex in the giant liposome as shown in Figure 4. The pore was formed in the upper left corner of the giant liposome. The CF was continuously released from this single pore without interruption. The kinetics of fluorescence loss from these liposomes is consistent with a 10–40 nm pore whose size is concentration dependent.[71] This suggests that the CF transporting Bax pore stayed open till the giant liposome is completely emptied or if there were some closures they would be very short so that CF release from the giant lipsome appeared continuous.

Conclusion

The role of Bcl-2-family proteins in programmed cell death is one of integration. The proapoptotic proteins function by integrating a large number of diverse afferent signals that are focused on the control of Bax and/or Bak to release cytochrome *c* from the mitochondrion. Our data indicate that Bax alone can form a cytochrome *c* competent pore in liposomes. The details of this activation include an in-membrane monomer, dimer and tetramer. This series of required protein–protein interactions offers a number of potential sites for proapoptotic augmentation and inhibition

by antiapoptotic proteins. The concept of a mitochondrial death decision point controlled by interaction between Bcl-2-family proteins was a view that Stan Korsmeyer arrived at early and promoted throughout his career. Stan invited me to participate in the study of these phenomena and was always intellectually generous in his support. We will all miss his further contributions.

References

1. Steller H (1995) Mechanisms and genes of cellular suicide. Science 267: 1445–1449.
2. Raff M (1992) Social controls on cell survival and cell death. Nature 356: 397–400.
3. Ellis R, Yuan J and Horvitz H (1991) Mechanisms and functions of cell death. Annu. Rev. Cell Biol. 7: 663–698.
4. Danial NN and Korsmeyer SJ (2004) Cell death: critical control points. Cell 116: 205–219.
5. Zimmermann KC, Bonzon C and Green DR (2001) The machinery of programmed cell death. Pharmacol. Therap. 92: 57–70.
6. Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ and Schlesinger PH (2000) Proapoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome *c*. Cell Death Differ. 7: 1166–1173.
7. Shi Y (2001) A structural view of mitochondria-mediated apoptosis. Nat. Struct. Biol. 8: 394–401.
8. Chao D and Korsmeyer S (1998) Bcl-2-family: regulators of cell death. Annu. Rev. Immunol. 16: 395–419.
9. Monaghan P, Robertson D, Amos T, Dyer M, Mason O and Greaves M (1992) Ultra-structural localization of bcl-2 protein. Histol. Cytochem. 40: 1819–1825.
10. Hockenbery D, Nunez G, Milliman C, Schreiber R and Korsmeyer S (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348: 334–336.
11. Adams J and Cory S (2001) Life-or-death decisions by the bcl-2 protein family. Trends Biochem. Sci. 26: 61–66.
12. Fesik SW (2000) Insights into programmed cell death through structural biology. Cell 103: 273–282.
13. Adams J and Cory S (1998) The bcl-2 protein family: arbiters of cell survival. Science 281: 1322–1326.
14. Kroemer G (1997) Mitochondrial implication in apoptosis. Towards an endosymbiont hypothesis of apoptosis evolution. Cell Death Differ. 4: 443–456.
15. Farrow S and Brown R (1966) New members of the bcl-2-family and their protein partners. Curr. Opin. Genet. Dev. 6: 45–49.
16. Cheng BH, Levine B, Boise LH, Thompson CB and Hardwick JM (1996) Baxindependent inhibition of apoptosis by bcl-xl. Nature 179: 554–556.
17. Yin XM, Oltvai Z and Korsmeyer S (1994) BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heteropolymerization with Bax. Nature 356: 321–323.
18. Sedlak T, Oltvai Z, Yang E, Wang K, Boise L, Thompson C and Korsmeyer S (1995) Multiple Bcl-2-family members demonstrate selective dimerizations with Bax. Proc. Natl. Acad. Sci. USA 92: 7834–7838.
19. Letai A, Bassik M, Walensky L, Sorcinelli M, Weiler S and Korsmeyer S (2002) Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell 2: 183–192.
20. Eskes R, Desagher S, Antonsson B and Martinou JC (2000) Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Mol. Cell. Biol. 20: 929–935.
21. Mikhailov V, Mikhailova M, Degenhardt K, Venkatachalam MA, White E and Saikumar P (2003) Association of Bax and Bak homo-oligomers in mitochondria: Bax requirement for Bak reorganization and cytochrome *c*release. J. Biol. Chem. 278: 5367–5376.
22. Mikhailov V, Mikhailova M, Pulkrabek DJ, Dong Z, Venkatachalam MA and Saikumar P (2001) Bcl-2 prevents Bax oligomerization in the mitochondrial outer membrane. J. Biol. Chem. 276: 18361–18374.
23. Gross A, Jockel J, Wei M and Korsmeyer S (1998) Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis. EMBO J. 17: 3878–3885.
24. Muchmore S, Sattler M, Liang H, Meadows R, Harlan J, Yoon H, Nettesheim D, Chang B, Thompson C, Wong S, Ng S and Fesik S (1996) X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature 381: 335–341.
25. Schlesinger P, Gross A, Xin X, Yamamoto K, Saito M, Waksman G and Korsmeyer S (1997) Comparison of the ion channel characteristics of proapoptotic bax and antiapoptotic bcl-2. Proc. Natl. Acad. Sci. USA 94: 11357–11362.
26. Antonsson B, Conti F, Giavatta A, Montessuit S, Lewis S, Martinou I, Bernasconi L, Bernard A, Mermod J, Mazzei G, Maundrell K, Gambale F, Sadoul R and Martinou J (1997) Inhibition of Bax channel-forming activity by Bcl-2. Science 277: 370–372.
27. Schendel S, Xie Z, Montal M, Matsuyama S, Montal M and Reed J (1997) Channel formation by antiapoptotic protein Bcl-2. Proc. Natl. Acad. Sci. USA 94: 5113–5118.
28. Minn A, Velez P, Schendel S, Liangli H, Muchmore S, Fesik S, Fill M and Thompsen C (1997) Bcl-xl forms an ion channel in synthetic lipid membranes. Nature 385: 353–356.
29. Jiang X and Wang X (2004) Cytochrome *c*-mediated apoptosis. Annu. Rev. Biochem. 73: 87–106.
30. Acehan D, X J, Morgan DG, Heuser JE, Wang X and Akey CW (2002) Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. Mol. Cell 9: 423–432.
31. Yang J, Liu X, Bhalla K, Kim C, Ibrado A, Cai J, Peng TI, Jones D and Wang X (1997) Prevention of apoptosis by bcl-2: release of cytochrome *c* from mitochondria blocked. Science 275: 1129–1132.
32. Liu X, Naekyung Kim C, Yang J, Jemmerson R and Wang X (1996) Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. Cell 86: 147–157.
33. Petros AM, Medek A, Nettesheim DG, Kim DH, Yoon HS, Swift K, Matayoshi ED, Oltersdorf T and Fesik SW (2001) Solution structure of the antiapoptotic protein Bcl-2. Proc. Natl. Acad. Sci. USA 98: 3012–3017.
34. Suzuki M, Youle RJ and Tjandra N (2000) Structure of Bax: coregulation of dimer formation and intracellular localization. Cell 103: 645–654.
35. Chou J, H L, Salvesen G, Yuan J and Wagner G (1999) Solution structure of BID, an intracellular amplifier of apoptotic signaling. Cell 96: 615–624.
36. McDonnell JM, Fushman D, Milliman CL, Korsmeyer SJ and Cowburn D (1999) Solution structure of the proapoptotic molecule BID: a structural basis for apoptotic agonists and antagonists. Cell 96: 625–634.
37. García-Sáez AJ, Coraiola M, Serra MD, Mingarro I, Menestrina G and Salgado J (2005) Peptides derived from apoptotic Bax and Bid reproduce the poration activity of the parent full-length proteins. Biophys. J. 88: 3976–3990.
38. Luo W, Yao X and Hong M (2005) Large structure rearrangement of colicin Ia channel domain after membrane binding from 2D ^{13}C spin diffusion NMR. J. Am. Chem. Soc. 127: 6402–6408.
39. Oh KJ, Barbuto S, Meyer N, Kim RS, Collier RJ and Korsmeyer SJ (2005) Conformational changes in BID, a pro-apoptotic BCL-2-family member, upon membrane binding: A site-directed spin labeling study. J. Biol. Chem. 280: 753–767.
40. Schendel SL, Montal M and Reed JC (1998) Bcl-2-family proteins as ion-channels. Cell Death Differ. 5: 372–380.
41. Zakharov SD and Cramer WA (2002) Colicin crystal structures: pathways and mechanisms for colicin insertion into membranes. Biochim. Biophys. Acta 1565: 333–346.
42. Perez D and White E (2000) TNF-α signals apoptosis through a Bid-dependent conformational change in Bax that is inhibited by E1B 19K. Mol. Cell 6: 53–63.
43. Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, Erdjument-Bromage H, Tempst P and Korsmeyer SJ (1999) Caspase cleaved BID targets mitochondria and is required for cytochrome *c* release, while BCL-Xl prevents this release but not tumor necrosis factor-R1/Fas death. J. Biol. Chem. 274: 1154–1163.
44. Zha J, Weiler S, Oh KJ, Wei MC and Korsmeyer SJ (2000) Posttranslational myristoylation of BID as a molecular switch for targeting mitochondria and apoptosis. Science 290: 1761–1765.
45. Wei MC, Lindsen T, Mootha VK, Weiler S, Gross A, Ashiya M, Thompson CB and Korsmeyer SJ (2000) tbid, a membrane targeted death ligand, binds and oligomerizes BAK to release cytochrome *c*. Genes Dev. 14: 2060–2071.

46. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T and Korsmeyer SJ (2001) BCL-2, BCL-XL sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol. Cell 8: 705–711.
47. Zha H, Fisk HA, Yaffe M, Mahajan N, Herman B and Reed J (1996) Structure–function comparisons of the proapoptotic protein Bax in yeast and mammalian cells. Mol. Cell. Biol. 16: 6494–6506.
48. Zha H, Aime-Sempe C, Sato T and Reed J (1996) Proapoptotic protein Bax heterodimerizes with Bcl-2 and homodimerizes with Bax via a novel domain (BH3) distinct from BHl and BH2. J. Biol. Chem. 271: 7440–7444.
49. Saito M, Korsmeyer SJ and Schlesinger PH (2000) Bax dependent cytochrome-*c* transport reconstituted in pure liposomes. Nat. Cell Biol. 2: 553–555.
50. Wei MC, Zong WX, Cheng EHY, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB and Korsmeyer SJ (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292: 727–730.
51. Sattler M, Liang H, Nettesheim D, Meadows R, Harlan J, Eberstadt M, Yoon H, Shuker S, Chang B, Minn A, Thompson C and Fesik S (1997) Structure of bcl-xl–bak peptide complex: recognition between regulators of apoptosis. Science 275: 983–986.
52. Wang K, Gross A, Waksman G and Korsmeyer SJ (1998) Mutagenesis of the BH3 domain of BAX identifies critical residues for dimerization and killing. Mol. Cell Biol. 18: 6089–6097.
53. Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL and Korsmeyer SJ (2004) Activation of apoptosis *in vivo* by a hydrocarbon-stapled BH3 helix. Science 305: 1466–1470.
54. Sundararajan R and White E (2001) E1B 19K blocks bax oligomerization and tumor necrosis factor alpha-mediated apoptosis. J. Virol. 75: 7506–7516.
55. Goping IS, Gross A, Lavoie JN, Nguyen M, Jemmerson R, Roth K, Korsmeyer SJ and Shore GC (1998) Regulated targeting of BAX to mitochondria. J. Cell Biol. 143: 207–215.
56. Nechushtan A, Smith CL, Hsu YT and Youle RJ (1999) Conformation of the Bax C-terminus regulates subcellular location and cell death. EMBO J. 18: 2330–2341.
57. Tzung SP, Kim KM, nez GB, Giedt CD, Simon J, Zimmerberg J, Zhang KYJ and Hockenbery DM (2001) Antimycin a mimics a cell-death-inducing Bcl-2 homology domain 3. Nat Cell Biol. 3: 183–191.
58. Degterev A, Lugovskoy A, Cardone M, Mulley B, Wagner G, Mitchison T and Yuan J (2001) Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL. Nat Cell Biol. 3: 173–184.
59. Losonczi JA, Olejniczak ET, Betz SF, Harlan JE, Mack J and Fesik SW (2000) NMR studies of the anti-apoptotic protein Bcl-xL in micelles. Biochemistry 39: 11024–11033.
60. Antonsson B, Montessuit S, Sanchez B and Martinou JC (2001) Bax is present as a high molecular weight oligomer/complex in the mitochondrial membrane of apoptotic cells. J. Biol. Chem. 276: 11615–11623.
61. Antonsson B, S Montessuit and SL, Eskes R and Martinou JC (2000) Bax oligomerization is required for channel-forming activity in liposomes and to trigger cytochrome *c* release from mitochondria. Biochem. J. 345: 271–278.
62. Rousseau F, Schymkowitz JWH and Itzhaki LS (2003) The unfolding story of three-dimensional domain swapping. Structure 11: 243–251.
63. Kundu S and Jernigan RL (2005) Molecular mechanism of domain swapping in proteins: an analysis of slower motions. Biophys. J. 86: 3846–3854.
64. Qin F, Auerbach A and Sach F (2000) A direct optimization approach to hidden Markov modeling for single channel kinetics. Biophys. J. 79: 1915–1927.
65. Dejean LM, Martinez-Caballero S, Guo L, Hughes C, Teijido O, Ducret T, Ichas F, Korsmeyer SJ, Antonsson B, Jonas EA and 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.
66. Martinez-Caballero S, Dejean LM, Jonas EA and Kinnally KW (2005) The role of the mitochondrial apoptosis induced channel mac in cytochrome *c* release. J. Bioenerg. Biomembr. 37: 155–164.
67. Martinez-Caballero S, Dejean LM and Kinnally KW (2004) Some amphiphilic cations block the mitochondrial apoptosis-induced channel, MAC. FEBS Lett. 568: 35–38.
68. Guo L, Pietkiewicz D, Pavlov EV, Grigoriev SM, Kasianowicz JJ, Dejean LM, Korsmeyer SJ, Antonsson B and Kinnally KW (2004) Effects of cytochrome *c* on the mitochondrial apoptosis-induced channel MAC. Am. J. Physiol. Cell. Physiol. 286: C1109–C1117.
69. Chen RF and Knutson JR (1988) Mechanism of fluorescence concentration quenching of carboxyfluorescein in liposomes: energy transfer to nonfluorescent dimers. Anal. Biochem. 172: 61–77.
70. Rex S and Schwarz G (1998) Quantitative studies on the melittin-induced leakage mechanism of lipid vesicles. Biochemistry 37: 2336–2345.
71. Miller C (1984) Ion channels in liposomes. Ann. Rev. Physiol. 46: 549–558.
72. Schwarz G, Zong R and Popescu T (1992) Kinetics of melittin induced pore formation in the membrane of lipid vesicles. Bioch. Biopys. Acta 1110: 97–104.
73. Segel IH (1975) *Enzyme Kinetics. Behavior and Analysis of Rapid Equilibrium and Steady-state Enzyme Systems*. John Wiley & SonsNew York:pp 371–375.
74. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR and Newmeyer DD (2002) Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 111: 331–342.
75. Cheng EHY, Sheiko TV, Fisher JK, Craigen WJ and Korsmeyer SJ (2003) VDAC2 inhibits BAK activation and mitochondrial apoptosis. Science 301: 513–517.
76. Roucou X, Montessuit S, Antonsson B and Martinou JC (2002) Bax oligomerization in mitochondrial membranes requires tBid (caspase-8-cleaved Bid) and a mitochondrial protein. Biochem. J. 368: 915.
77. Epand RF, Martinou JC, Montessuit S and Epand RM (2004) Fatty acids enhance membrane permeabilization by proapoptotic Bax. Biochem. J. 377: 509–516.
78. Kim S and Martin GM (1981) Preparation of cell-sized unilamellar liposomes with high capture volume and defined size distribution. Bioch. Biophys. Acta 646: 1–9.