



Review

The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability

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Abstract

Mitochondria are well known as sites of electron transport and generators of cellular ATP. Mitochondria also appear to be sites of cell survival regulation. In the process of programmed cell death, mediators of apoptosis can be released from mitochondria through disruptions in the outer mitochondrial membrane; these mediators then participate in the activation of caspases and of DNA degradation. Thus the regulation of outer mitochondrial membrane integrity is an important control point for apoptosis. The Bcl-2 family is made up of outer mitochondrial membrane proteins that can regulate cell survival, but the mechanisms by which Bcl-2 family proteins act remain controversial. Most metabolites are permeant to the outer membrane through the voltage dependent anion channel (VDAC), and Bcl-2 family proteins appear to be able to regulate VDAC function. In addition, many Bcl-2 family proteins can form channels *in vitro*, and some pro-apoptotic members may form multimeric channels large enough to release apoptosis promoting proteins from the intermembrane space. Alternatively, Bcl-2 family proteins have been hypothesized to coordinate the permeability of both the outer and inner mitochondrial membranes through the permeability transition (PT) pore. Increasing evidence suggests that alterations in cellular metabolism can lead to pro-apoptotic changes, including changes in intracellular pH, redox potential and ion transport. By regulating mitochondrial membrane physiology, Bcl-2 proteins also affect mitochondrial energy generation, and thus influence cellular bioenergetics. *Cell Death and Differentiation* (2000) 7, 1182–1191.

Keywords: apoptosis; Bcl-x_L; Bcl-2; Bax; mitochondria; outer mitochondrial membrane

Abbreviations: ANT, adenine nucleotide translocator; OMM, outer mitochondrial membrane; ROS, reactive oxygen species; VDAC, voltage dependent anion channel

Introduction

Mitochondria became the subject of intensive scientific investigation in the mid-twentieth century when their role as cellular energy producers was discovered. This culminated in the discovery and characterization of oxidative phosphorylation as the mechanism by which most cellular ATP is produced. Free exchange of substrate and ATP/ADP between mitochondria and the cytosol was proposed to provide the homeostatic mechanism by which glycolysis and oxidative phosphorylation are coupled to maintain the intracellular ATP/ADP ratio.^{1,2} In the last few years, there has been a resurgence of interest in mitochondria following the discovery that mitochondria play a crucial role in the regulation of programmed cell death, or apoptosis.^{3–9} A number of molecules involved in the execution of apoptosis normally reside in mitochondria, safely sequestered from their targets and co-factors.^{10–16} Following an apoptotic stimulus, these proteins can be released from mitochondria and initiate the activation of the downstream effectors of apoptosis, caspases. The mechanisms by which these apoptosis initiators are released from mitochondria remain controversial. Elucidating these mechanisms, however, is crucial to the understanding of how and why a cell makes the decision to undergo apoptosis.

The mitochondrion is an organelle encompassed by two membranes (Figure 1). The inner membrane, which surrounds the mitochondrial matrix and is usually tightly folded in cristae, holds the molecular complexes of the electron transport chain. It is across the inner membrane that the electron transport chain generates the hydrogen ion gradient necessary to make ATP. In order to maintain this crucial gradient, transport across the inner membrane is tightly regulated by many different, specific transporters for the metabolites that need to cross the inner membrane, such as the phosphate carrier, the dicarboxylate carrier and the tricarboxylate carrier.¹⁷ The inner membrane also contains protein import channels, as well as the adenine nucleotide translocator (ANT) that exchanges ADP and ATP between the mitochondrial matrix and the intermembrane space. The outer mitochondrial membrane (OMM) surrounds the inner membrane, creating the intermembrane space where many of the characterized apoptosis promoting proteins reside, including cytochrome *c*, apoptosis inducing factor (AIF) and some pro-caspases. Transport across the outer mitochondrial membrane is thought to be less tightly regulated than transport across the inner membrane. The high permeability of the outer membrane is mediated by VDAC, which is the most common protein in the outer membrane and is permeable to molecules of up to 5000 daltons in its open configuration.¹⁸ Nevertheless, the outer membrane is impermeable to holo-cytochrome *c* and other apoptosis promoting proteins in the intermem-

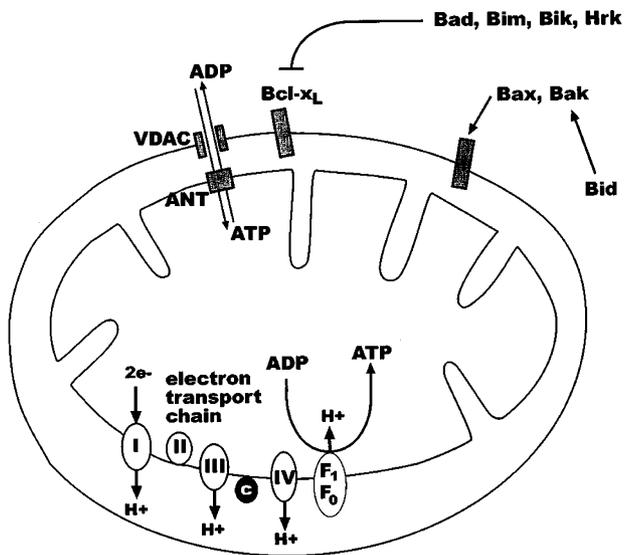


Figure 1 Mitochondria are producers of ATP and regulators of apoptosis. Oxidative phosphorylation occurs across the mitochondrial inner membrane via the electron transport chain, including cytochrome *c* (*c*), and the F₁F₀-ATPase (F₁F₀). ATP made in the matrix is exchanged for cytosolic ADP through the adenine nucleotide translocator (ANT) in the inner membrane and the voltage dependent anion channel (VDAC) in the outer membrane. Bcl-2 family proteins regulate mitochondrial physiology and apoptosis. Bcl-x_L is constitutively localized to the outer membrane and may be inhibited through interactions with BH3-only proteins including Bad, Bim, Bik and Hrk. Bax translocates to the outer membrane after an apoptotic signal and can be toxic in the absence of a physical interaction with Bcl-x_L.

brane space and this impermeability is crucial to the regulation of apoptosis. For example, if holo-cytochrome *c* is released into the cytosol, it can complex with Apaf-1 and ATP or dATP to activate pro-caspase 9, thus beginning the proteolytic cascade that results in the morphological features of apoptosis.¹⁹

Maintenance of the permeability of the outer membrane to metabolic anions is crucial for cell survival. Free ATP/ADP exchange, as well as the movement of other metabolic anions such as creatine phosphate, succinate and pyruvate between the cytosol and the matrix, is dependent on passage through the outer membrane, primarily through VDAC.^{20,21} Paradoxically, the continued exchange of metabolic anions across the outer membrane is required to maintain the integrity of this membrane and to prevent the release of apoptosis promoting proteins. Decreased passage of metabolites across the membrane would tend to favor inner membrane hyperpolarization, increased reactive oxygen species (ROS) production and matrix swelling, all of which could compromise the integrity of the outer membrane.

Bcl-2 family

Members of the Bcl-2 family of proteins may regulate OMM integrity and function. Bcl-2 proteins can localize to the outer membrane and are established regulators of apoptosis.^{5,6,22} The family can be divided into three different groups based on Bcl-2 homology (BH) domains and function.²³ The anti-

apoptotic members, such as Bcl-2 and Bcl-x_L, typically have BH1 through BH4 domains. The pro-apoptotic members can be divided into two groups: those with BH1, BH2 and BH3 domains, such as Bax and Bak, and those with only BH3 domains, such as Bad, and Bim.^{24–26} An exception to these categories may be Bcl-x_S, a pro-apoptotic protein, which is an alternate splice form of Bcl-x_L and contains BH3 and BH4 domains.²⁷ These domains have functional and structural significance. The BH3 domain of pro-apoptotic family members can interact with the hydrophobic cleft formed by the BH1, BH2 and BH3 domains of anti-apoptotic proteins, and this interaction may be an important regulatory mechanism.^{28,29} Structural studies of Bcl-x_L have shown a striking similarity between Bcl-x_L and the colicin family of pore forming proteins,³⁰ and functional studies have shown that Bcl-x_L, as well as Bcl-2, Bax, and Bak, are capable of forming channels in synthetic lipid bilayers.^{31–34} This functional property of Bcl-2 family proteins may be even more significant than originally appreciated, since it was recently shown that cleaved Bid, which shares primary sequence homology with Bcl-x_L only in the BH3 domain, has a similar three-dimensional structure to Bcl-x_L and can also form channels in synthetic lipid bilayers.^{35–37} The ability of Bcl-2 family members to form channels in association with intracellular membranes appears to be important to their function in apoptosis, as does their ability to localize to the OMM.^{22,29} Thus a number of groups have sought to determine whether Bcl-2 family proteins may perform their functions by regulating the permeability of the outer mitochondrial membrane. Furthermore, by regulating the permeability of the outer mitochondrial membrane, Bcl-2 family members could potentially be regulating mitochondrial homeostasis in general, rather than being solely involved in apoptosis regulation.

The mechanisms by which the outer mitochondrial membrane increases in permeability during apoptosis, and the mechanisms by which Bcl-2 proteins regulate this process have remained controversial. A number of models for outer membrane permeabilization have been proposed, such as the non-specific rupture of the outer membrane or the formation of a specific channel. Bcl-2 family proteins may regulate both of these processes, via both physical interactions and channel forming capabilities. In any case, it is clear that the disruption in the OMM during apoptosis must be large and freely permeable, since every intermembrane space molecule that has been measured appears to pass through the channel or hole that ultimately forms.³⁸

Non-specific outer membrane rupture

Non-specific rupture of the outer mitochondrial membrane is one possible mechanism for the increase in outer membrane permeability following an apoptotic stimulus. This is an attractive model to explain the non-selective release of intermembrane space proteins and the simultaneous accessibility of the inner membrane to large cytosolic proteins. Rupture of the outer mitochondrial membrane could be secondary to swelling of the matrix, which can expand to a greater surface area than the outer membrane due to the

extensive folding of the inner membrane. Mitochondrial swelling has been reported to occur following many apoptotic stimuli, including growth factor withdrawal, heat shock, sustained increase in intracellular calcium levels, TNF treatment and ischemia.^{39–44} Matrix swelling that leads to outer membrane rupture could occur in at least two ways. One model hypothesizes that different apoptotic stimuli have downstream metabolic effects on mitochondria that induce matrix swelling, while another model invokes PT pore opening as the cause of swelling.

Matrix swelling and OMM rupture in response to metabolic changes

Matrix swelling is known to occur in response to a reduction in the rate of electron transport.⁴⁵ A reduction in the rate of electron transport can occur in the presence of mitochondrial poisons, or due to mitochondrial substrate limitation,³⁹ which can occur when a cell has inadequate nutrients, oxygen, or cannot transport substrates into the mitochondria.⁴⁶ These conditions are associated with the induction of apoptosis in multiple systems.^{47,48}

Matrix swelling necessarily leads to a reduction in intermembrane space volume. The intermembrane space contains a number of proteins that are impermeant to the outer membrane. Because these proteins cannot equilibrate across the outer membrane, maintaining a chemical equilibrium in the face of intermembrane space volume changes could involve the movement of ions, thus generating an electrical gradient across the outer membrane (Donnan potential). This electrical gradient would favor closure of VDAC, which could exacerbate the initial swelling. By limiting exchange of ADP for ATP across the outer membrane, VDAC closure would stall the electron transport chain by limiting the ability of the ATP synthase to utilize the hydrogen ion gradient to generate ATP from ADP. Under such conditions, osmotic influx of weak organic acids will lead to slow swelling of the matrix. Eventually, as the mitochondria become more polarized, and the rate of electron transport slows, the matrix space would be predicted to swell beyond the confines of the outer membrane, resulting in outer membrane rupture and the release of pro-apoptotic proteins into the cytosol. Tears in the outer mitochondrial membrane have been observed by electron microscopy in response to growth factor withdrawal.^{39,49} An ability to maintain VDAC in an open configuration despite a charge across the outer membrane or an ability to prevent the build-up of charge across the outer membrane would allow the outer membrane to withstand the stress associated with matrix swelling, and it has been suggested that anti-apoptotic Bcl-2 proteins may play such a role.⁵⁰

Consistent with this model, it has been demonstrated that following growth factor withdrawal, there is a build-up of ADP in the cytosol, and a build-up of ATP in the mitochondria.⁴⁸ These conditions could result from a decrease in exchange between the mitochondria and the cytosol. In addition, mitochondria also accumulate creatine phosphate following growth factor withdrawal.

Creatine phosphate is made in the intermembrane space from ATP and creatine in a reaction requiring creatine kinase. Creatine phosphate then diffuses into the cytosol to buffer falling ATP levels.⁵¹ The accumulation of creatine phosphate in mitochondria therefore suggests that transport across the OMM is impaired, while the continued production of creatine phosphate implies that ATP transport across the inner membrane is intact.²¹ The defect in exchange across the OMM, with a subsequent stalling of the F_1F_0 -ATPase's utilization of the hydrogen ion gradient, could also be responsible for the mitochondrial hyperpolarization that has been reported in response to multiple apoptotic stimuli.^{29,39,52,53} In addition, reduced function of the ATP synthase could lead to increased ROS production and oxidation of outer membrane lipids.

Anti-apoptotic Bcl-2 proteins prevent disruption of mitochondrial metabolism

Anti-apoptotic Bcl-2 family members have been shown to prevent the matrix swelling, ROS damage, release of cytochrome c and loss of membrane potential associated with apoptosis.^{39,54–56} They have also been shown to prevent the disruption in ATP/ADP exchange between the mitochondrial matrix and the cytosol.⁴⁸ The defect in exchange has been localized to the outer mitochondrial membrane where both VDAC and Bcl-2 family proteins are localized.²¹ Therefore, one way Bcl-2 family proteins could modulate ATP/ADP exchange is through regulation of VDAC (Figure 2). VDAC can exist in both open and closed conformations. In closed conformations, VDAC is impermeable to ATP and other metabolic anions (Figure 2A).^{21,57} By maintaining VDAC in an open conformation, Bcl-2 family proteins would be able to maintain ATP/ADP exchange, thus preventing mitochondrial hyperpolarization, swelling and rupture (Figure 2B).

Recent *in vitro* data demonstrate that recombinant Bcl-x_L can maintain VDAC in an open conformation. Studies in planar phospholipid membranes showed that when physiologic voltages of between –25 and +25 mV are applied to a membrane containing VDAC, addition of rBcl-x_L favors the maintenance of VDAC in its high conductance state. In addition, isolated mouse liver mitochondria in the presence of a VDAC inhibitor (β -NADH) consumed ADP at a faster rate after rBcl-x_L addition, a process requiring more efficient transport of ADP across the outer membrane. Finally, mitochondria isolated from growth factor withdrawn cells had accumulated creatine phosphate due to a defect in outer membrane permeability. These mitochondria were able to release creatine phosphate, but not cytochrome c, in response to added rBcl-x_L.⁵⁸ Together, these data suggest that Bcl-x_L can maintain VDAC in the open state. How Bcl-x_L maintains the open state of VDAC is unclear. Bcl-x_L could form a direct physical interaction with VDAC, or Bcl-x_L insertion could maintain the VDAC open state by affecting the local lipid concentration or distribution. Alternatively, Bcl-x_L could dissipate charge on the outer membrane by acting as a channel and thus indirectly affect

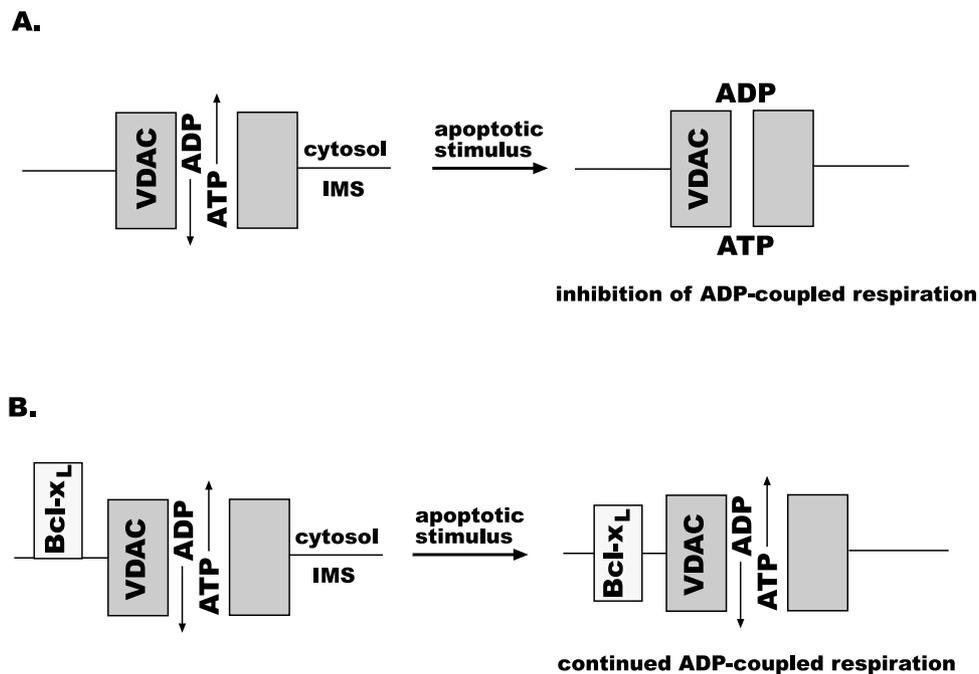


Figure 2 Bcl-x_L regulates outer membrane permeability via regulation of VDAC. (A) Under normal conditions, VDAC exchanges metabolic anions, including ATP and ADP, between the cytosol and the intermembrane space (IMS). In the absence of Bcl-x_L, apoptotic signals can lead to VDAC closure, which disrupts the transport of metabolic anions across the outer mitochondrial membrane. This leads to mitochondrial dysfunction, including swelling, which can eventually result in outer membrane rupture and cytochrome *c* release. (B) The presence of Bcl-x_L at the outer membrane during apoptotic signal transduction allows for maintenance of VDAC in the open configuration and prevention of mitochondrial dysfunction and apoptosis

VDAC. However Bcl-x_L functions to maintain the open state of VDAC, this property would sustain metabolite exchange and prevent rupture of the outer mitochondrial membrane and release of apoptosis promoting proteins.

Pro-apoptotic Bcl-2 proteins disrupt mitochondrial metabolism

The pro-apoptotic Bcl-2 family members themselves may be capable of inducing the mitochondrial transport problems that can lead to matrix swelling. Many of these proteins have been shown to change conformation and translocate to the mitochondrial membrane in response to various apoptotic stimuli. Some data suggests that there may be a lag time between Bax translocation and cell death,⁵⁹ suggesting that the pro-apoptotic family members may not always release cytochrome *c* directly, but rather may contribute to a mitochondrial dysfunction that leads to cytochrome *c* release. A direct impact of Bax on membrane permeability was suggested by recent studies in the yeast *S. cerevisiae*, where a mutant impaired in transport across the outer mitochondrial membrane due to a deficiency of yeast VDAC was more susceptible than wild-type yeast to Bax induced toxicity.⁶⁰ This suggests that Bax and VDAC may have opposing functions on the permeability of the outer membrane. Bax over-expression in *S. cerevisiae* has been shown to induce mitochondrial hyperpolarization and metabolic arrest, which is also suggestive of a block in substrate transport or in the activity of the F₁F₀-ATPase.⁵³

Other groups, however, have shown that Bax and Bid are capable of promoting cytochrome *c* release in the absence of observed matrix swelling.³⁸ These data, along with data demonstrating the ability of Bax to multimerize and form larger membrane channels, suggests that Bax may directly induce cytochrome *c* releasing channels in the mitochondrial outer membrane without prior alteration in mitochondrial physiology.

Reactive oxygen species

The redox state of a cell has been shown to play an important role in many types of apoptosis, including death induced by TNF, UV, growth factor withdrawal and ceramide.⁶¹ Bcl-2 and Bcl-x_L expression can protect cells from oxidant-induced apoptosis, although it has remained unclear whether the protective effect is due to a scavenging function or to a regulation of the production of ROS.^{54,56,61} How does this function of Bcl-2 family proteins tie in with Bcl-2 family regulation of outer mitochondrial membrane permeability? Mitochondrial ROS can be produced when there is a backup in the electron transport chain, particularly a block after complex III, which is thought to produce most mitochondrial ROS. As described above, a decrease in the permeability of the outer mitochondrial could cause a backup in the electron transport chain as ADP transport into the mitochondria becomes limiting. Therefore, a block in mitochondrial transport of ADP could lead to mitochondrial hyperpolarization as well as production of ROS. Bcl-x_L can restore outer mitochondrial membrane permeability, thus relieving the

block in electron transport and preventing both ROS production and hyperpolarization. Hyperpolarization in combination with ROS production has been seen in response to Fas signaling and Bax over-expression.^{53,62} Mitochondrial hyperpolarization may be unstable and progress to a loss in membrane potential, which can lead to further ROS production. Recent work has shown that in response to treatment with TNF- α , 2B4 cells, a murine T-cell hybridoma cell line, show a decreased mitochondrial membrane potential that is accompanied by ROS production. Bcl-x_L expression prevented both the drop in membrane potential and the increase in ROS production while delaying the kinetics of apoptosis. However Bcl-x_L was not acting as a scavenger since the basal levels of ROS production were the same in both Bcl-x_L expressing and control cells.⁶³ These data suggest that Bcl-x_L is able to both attenuate ROS production and delay apoptosis by regulating mitochondrial physiology. Further work will be required to understand the etiology and the impact of the ROS induced during apoptosis.

Matrix swelling and OMM rupture in response to PT pore opening

Opening of the permeability transition (PT) pore is another model that postulates that outer membrane rupture is responsible for cytochrome *c* release (Figure 3).^{64,65} The PT pore is proposed to be a large multi-protein complex that can form a large channel between the cytosol and the mitochondrial matrix, with the ability to pass proteins up to 1.5 kD, in response to a number of mitochondrial stresses.⁶⁶ Opening of the PT pore correlates with swelling of the matrix and eventual rupture of the outer mitochondrial membrane with the release of cytochrome *c* and other proteins from the intermembrane space. Proposed components of the PT pore include VDAC, the ANT, creatine kinase, hexokinase, the benzodiazapine receptor and cyclophilin D.^{66–68} Molecules that bind different components of the PT pore, such as cyclosporin A, bongkreic acid, or Bcl-2 family members themselves, may regulate pore function.⁶⁹ These compounds also prevent apoptosis in response to some stimuli, indicating that opening of the PT pore may be an early event in apoptosis.^{64,69} A model in which opening of the PT pore

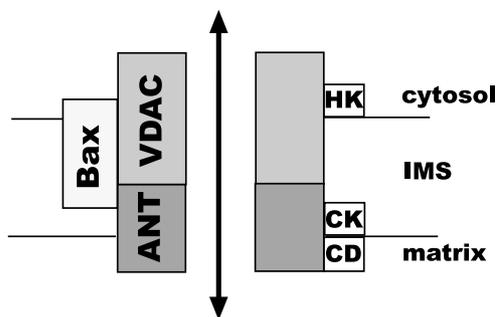


Figure 3 The proposed permeability transition pore. The PT pore is made up of a number of mitochondrial proteins and can be regulated by Bcl-2 proteins. Opening of the pore requires interactions between VDAC, the ANT, hexokinase (HK), creatine kinase (CK) and cyclophilin D (CD). Bax may regulate opening of the pore via an interaction with the ANT. Opening of the pore leads to equilibration between the matrix and the cytosol

commits the cell to apoptosis would predict an initial increase in the passage of molecules between the mitochondrial matrix and the cytosol. In fact, some reports have shown that large molecules previously impermeant to mitochondria do gain access to the matrix.⁷⁰ Another prediction of the PT pore model is a rapid depolarization of the inner membrane as the pore opens. While depolarization eventually does occur in all apoptosing cells, its role as an initiating event is called into question by multiple reports of mitochondrial hyperpolarization and subsequent matrix alkalinization as an early event in apoptosis.^{29,39,52,53,71} Furthermore, studies done with FL5.12 cells have shown that cells that have undergone hyperpolarization are doomed to die.⁴⁸ Hyperpolarization could precede the opening of the PT pore, but this would place hyperpolarization rather than depolarization at the commitment point to apoptosis.

Release of pro-apoptotic proteins through specific OMM channels

Another mechanism for the release of cytochrome *c* and other pro-apoptotic proteins from the mitochondrial intermembrane space is the formation of specific channels in the OMM. These channels must be large enough to release intermembrane space proteins non-specifically. One way these channels have been hypothesized to form is through interactions between Bax and VDAC to form hybrid channels. Another possibility is the multimerization of pro-apoptotic proteins to form a single large channel.

Specific channel formed by Bax and the voltage-dependent anion channel

One model for the disruption of OMM permeability by specific channels postulates that VDAC and Bax together can form a hybrid channel capable of releasing cytochrome *c* (Figure 4). Electrophysiology studies on VDAC and Bax suggest that together these proteins can form a channel with a larger pore size than either VDAC or Bax alone.⁷² In support of the hypothesis that this interaction between Bax and VDAC is required for Bax activity, experiments using mitochondria isolated from *S. cerevisiae* deficient in yeast VDAC showed that these mitochondria did not release cytochrome *c* in response to Bax.⁷³ Other studies, however, have shown that VDAC-deficient yeast are at least as sensitive, if not more sensitive, to Bax toxicity as wild-type yeast.^{53,60} Thus, the observed interaction between Bax and VDAC does not appear to be required for Bax toxicity *in vivo*.

This model also postulates regulation of VDAC by Bcl-x_L, but attributes a different effect of this regulation on OMM permeability. In this case, Bcl-x_L closes VDAC, preventing the release of cytochrome *c*. In a recent study, co-immunoprecipitation experiments identified VDAC as a Bcl-x_L-interacting protein. Further experiments tested the sucrose uptake of synthetic liposomes containing either VDAC alone, VDAC with rBcl-x_L or VDAC with rBax. Liposomes with VDAC alone took up sucrose, whereas those with both VDAC and rBcl-x_L were not able to do so and those with VDAC and rBax showed enhanced sucrose uptake. Furthermore, although liposomes with either VDAC

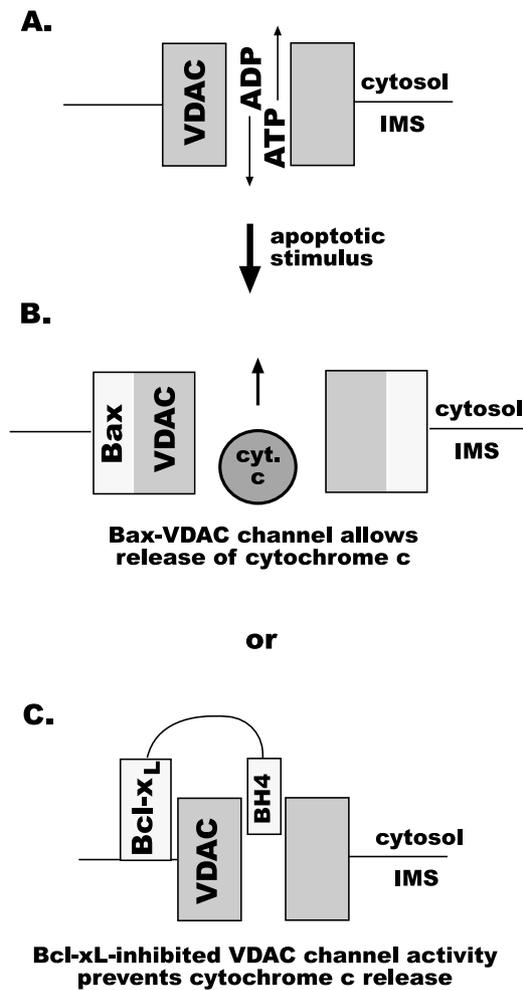


Figure 4 Bcl-2 family proteins may induce novel open and closed states of VDAC. (A) In the absence of Bcl-2 family regulation, VDAC functions normally. (B) Bcl-x_L can physically interact with VDAC, which leads to VDAC closure via interactions between VDAC and the BH4 domain. (C) Bax and VDAC together form a novel hybrid channel with the capacity to release cytochrome *c*

alone or Bax alone were not permeable to cytochrome *c*, the addition of rBax or rBak to VDAC-containing liposomes resulted in cytochrome *c* release.⁷³

Electrophysiology experiments to evaluate Bcl-x_L and VDAC interaction in bilayers support the hypothesis that Bcl-x_L can close VDAC.⁷² These results are different from the results described above suggesting that Bcl-x_L can maintain VDAC in the open configuration.⁵⁸ The differences between the findings of the groups that have performed electrophysiology studies on VDAC and Bcl-x_L may be due to the disparity in the voltages applied to the membranes. The study by Vander Heiden *et al.*⁵⁸ focused on potentials between +25 and -25 mV, while the study by Shimizu *et al.* typically used a voltage of +30 mV. The potential difference attained across the outer membrane preceding apoptosis *in vivo* is not known, although the outer membrane is thought to carry little or no voltage under most conditions, and transmembrane voltages of 20 mV or more are usually sufficient to close VDAC *in vitro*.^{74,75}

Additional work has shown that the BH4 domain alone of Bcl-2 or Bcl-x_L is capable of inhibiting VDAC-dependent cytochrome *c* release.⁷⁶ It is interesting to consider this result in light of previous results indicating that caspase cleavage of Bcl-2 or Bcl-x_L yields both a BH3 containing pro-apoptotic protein that localizes to mitochondria and a BH4 containing peptide.^{77,78} Also, Bcl-x_S, a pro-apoptotic protein, contains a BH4 domain, so the possession of a BH4 domain alone may not be the sole determinant of anti-apoptotic activity.

It is difficult to reconcile the concept of the pro-survival protein Bcl-x_L closing VDAC with the physiologic necessity of metabolite transport across the outer membrane. Perhaps this effect is only transient, or only affects a small proportion of VDAC molecules at once. On the other hand, it is easier to see how facilitation of the continued transport of metabolites by Bcl-x_L could promote survival. Although the differences between these models remain to be worked out, the regulation of VDAC, and thus of outer membrane permeability, by Bcl-2 family proteins seems likely to play a role in the regulation of cell survival.

Specific OMM channels formed by pro-apoptotic Bcl-2 protein

Another possible mechanism for the disruption in permeability of the outer mitochondrial membrane during apoptosis is the formation of large channels by pro-apoptotic Bcl-2 family members themselves (Figure 5).⁷⁹ Bcl-2 family members have been shown to be capable of inserting into the outer mitochondrial membrane, and presumably then function at this subcellular location. The anti-apoptotic members typically reside on the outer mitochondrial membrane all of the time. Some pro-apoptotic members, however, normally reside in the cytosol, and translocate to the outer membrane in response to various stress signals.⁸⁰⁻⁸²

The pro-apoptotic Bcl-2 family member that has been most studied in this regard is Bax. Under normal cellular conditions, Bax is found in the cytosol as a monomer,^{59,83} although there have also been reports that Bax is constitutively localized to the outer mitochondrial membrane.⁸⁴ After an apoptotic stimulus, however, Bax has been shown to change its conformation to a pro-apoptotic form and to translocate to the outer mitochondrial membrane,^{82,83} perhaps as a result of interactions with Bid or pH changes.^{59,84} This change in conformation leads to the exposure of the N-terminal domain and the C-terminal transmembrane domain. These conformational studies have been performed by using mutation studies and antibodies to specific regions of the protein as well as by assessing protease sensitivity.^{59,81,82,84,85} It has also been shown using enforced dimerization and cross-linking that, while in the cytosol Bax exists as a monomer, in its pro-apoptotic form in the outer membrane, Bax may exist in an oligomer.⁸⁶ Furthermore, multimerization has been shown to be sufficient for Bax translocation.⁸³ Oligomeric Bax has been shown to be capable of releasing cytochrome *c* from isolated mitochondria under conditions in which monomeric Bax was unable to do so.⁸⁷

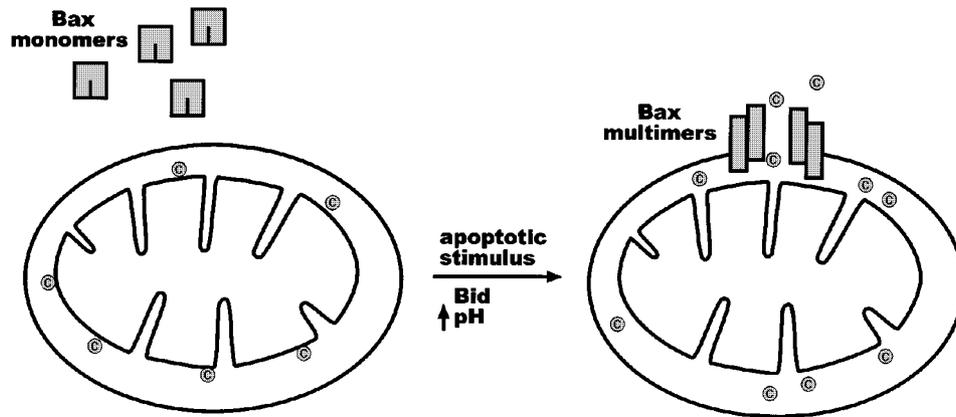


Figure 5 Bax forms multimeric complexes capable of releasing cytochrome *c* (c). In response to apoptotic signals such as cleaved Bid or a rise in pH, cytosolic Bax can multimerize and form large channels at the mitochondrial outer membrane. The subsequent cytochrome *c* release may not be accompanied by matrix swelling

Thus, these data suggest that in response to a cellular stress, Bax changes conformation and then inserts into the outer mitochondrial membrane where it is capable of forming multimeric channels large enough to release cytochrome *c* and other apoptosis promoting proteins. In this model, protection by anti-apoptotic Bcl-2 family members could be mediated by binding to Bax and thus preventing Bax-complex formation, or by changing the dynamics of the outer mitochondrial membrane in such a way as to disfavor the formation of Bax complexes or insertion. A model in which Bcl-x_L is not required to bind to Bax in order to protect from cell death may be more likely because Bcl-x_L has been shown to retain its protective effects in the absence of physical interactions with Bax.²⁹

The BH3-only proteins also play a significant role in the regulation of outer mitochondrial membrane permeability. Addition of recombinant Bid to isolated mitochondria has been shown to induce cytochrome *c* release³⁸ as well as Bax oligomerization and insertion.⁸⁶ Bid can be cleaved by caspase 8 *in vivo* after caspase 8 activation following death receptor stimulation. This allows for mitochondrial amplification of the apoptotic cascade after ligation of Fas or TNF receptors. Also, other BH3 family proteins, such as Bad, Bim, Harakiri and Bik, can translocate to mitochondria and bind to anti-apoptotic Bcl-2 family proteins in response to various apoptotic stimuli.^{25,88–90}

Conclusion

Much progress has been made in the field of mitochondrial regulation of apoptosis since the observation that mitochondria were required for apoptotic activity in cell extracts.^{8,9} Progress has also been made in the field of Bcl-2 family regulation of mitochondria since the observation that Bcl-2 proteins localize to the outer mitochondrial membrane.^{3,5–7} It is now known that mitochondrial release of cytochrome *c* is a crucial event in caspase activation and that anti-apoptotic Bcl-2 proteins can prevent this release while pro-apoptotic family members can promote release. Mitochondria are crucial for

eukaryotic life and severe mitochondrial damage invariably leads to death. In addition to preventing cytochrome *c* release, anti-apoptotic Bcl-2 proteins are able to maintain mitochondrial homeostasis, preventing the kind of damage to mitochondria that will kill a cell even when the apoptotic cascade is disabled. Furthermore, pro-apoptotic Bcl-2 proteins can kill eukaryotic cells in the absence of an apoptotic cascade.^{91,92}

Bcl-2 family proteins have recently been shown to regulate the integrity of the mitochondrial outer membrane via regulation of the outer membrane protein VDAC. The outer membrane has not been appreciated as a regulatory site for mitochondrial energy generation, but a change in its permeability in either direction has the potential to cause severe perturbations of mitochondrial physiology. If the outer membrane loses permeability after VDAC closure, metabolic anions such as ADP and malate will no longer be available to the mitochondria. This would slow down processes like ATP synthesis, the citric acid cycle and NAD⁺/NADH shuttles, leading to falling ATP and NAD⁺ levels, adversely affecting the energetic capacity of the cell, as well as leading to a drop in CO₂ levels, which could affect the pH balance of the cell. These perturbations in mitochondrial physiology will eventually lead to increased ROS production and damage to membranes, swelling and rupture of the outer membrane with concomitant release of cytochrome *c*. A large increase in outer membrane permeability would lead directly to cytochrome *c* release, which could decrease the efficiency of the electron transport chain in addition to activating the apoptotic cascade.

There is now evidence that Bcl-2 family proteins can participate in the regulation of outer membrane permeability. Bcl-x_L has been shown to regulate VDAC in its open configuration under conditions that would normally result in VDAC closure. Bax may be able to cause a decrease in permeability of the outer membrane in some conditions, resulting in sensitivity to VDAC deficiency, ROS production and mitochondrial hyperpolarization. In other conditions, Bax appears to form large pores in the outer membrane

capable of releasing cytochrome *c*. The regulation of outer mitochondrial membrane permeability is clearly a complex issue with wide-ranging effects on cellular physiology, impacting both cell survival decisions and bioenergetic capacity.

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References

- Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic try of mechanism. *Nature* 191: 144–148
- Ernster L and SCHATZ G (1981) Mitochondria: a historical review. *J. Cell. Biol.* 91: 227S–255S
- Chen-Levy Z and Cleary ML (1990) Membrane topology of the Bcl-2 proto-oncogenic protein demonstrated in vitro. *J. Biol. Chem.* 265: 4929–4933
- Hockenbery D, Nunez G, Milliman C, Schreiber RD and Korsmeyer SJ (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 348: 334–336
- Nguyen M, Millar DG, Yong VW, Korsmeyer SJ and Shore GC (1993) Targeting of Bcl-2 to the mitochondrial outer membrane by a COOH-terminal signal anchor sequence. *J. Biol. Chem.* 268: 25265–25268
- Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W and Reed JC (1993) Investigation of the subcellular distribution of the Bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes. *Cancer Res.* 53: 4701–4714
- Lithgow T, van Driel R, Bertram JF and Strasser A (1994) The protein product of the oncogene BCL-2 is a component of the nuclear envelope, the endoplasmic reticulum, and the outer mitochondrial membrane. *Cell Growth Differ.* 5: 411–417
- Newmeyer DD, Farschon DM and 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
- Liu X, Kim CN, 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
- Susin SA, Lorenzo HK, Zamzami N, Marzo I, Brenner C, Larochette N, Prevost MC, Alzari PM and Kroemer G (1999) Mitochondrial release of caspase-2 and -9 during the apoptotic process. *J. Exp. Med.* 189: 381–394
- Mancini M, Nicholson DW, Roy S, Thornberry NA, Peterson EP, Casciola-Rosen LA and Rosen A (1998) The caspase-3 precursor has a cytosolic and mitochondrial distribution: implications for apoptotic signaling. *J. Cell. Biol.* 140: 1485–1495
- Samali A, Zhivotovsky B, Jones DP and Orrenius S (1998) Detection of pro-caspase-3 in cytosol and mitochondria of various tissues. *FEBS Lett.* 431: 167–169
- Krajewski S, Krajewska M, Ellerby LM, Welsh K, Xie Z, Deveraux QL, Salvesen GS, Bredesen DE, Rosenthal RE, Fiskum G and Reed JC (1999) Release of caspase-9 from mitochondria during neuronal apoptosis and cerebral ischemia. *Proc. Natl. Acad. Sci. USA* 96: 5752–5757
- Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Abersold R, Siderovski DP, Penninger JM and Kroemer G (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397: 441–446
- Kluck RM, Bossy-Wetzel E, Green DR and Newmeyer DD (1997) The release of cytochrome *c* from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 275: 1132–1136
- Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP and Wang X (1997) Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked. *Science* 275: 1129–1132
- Stryer L (1995) *Biochemistry*, fourth edition, WH Freeman, New York, p.531
- Mannella CA (1992) The 'ins' and 'outs' of mitochondrial membrane channels. *Trends Biochem. Sci.* 17: 315–320
- Zou H, Henzel WJ, Liu X, Lutschg A and 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
- Hodge T and Colombini M (1997) Regulation of metabolite flux through voltage-gating of VDAC channels. *J. Membr. Biol.* 157: 271–279
- Vander Heiden MG, Chandel NS, Li XX, Schumacker PT, Colombini M and Thompson CB (2000) Outer mitochondrial membrane permeability can regulate coupled respiration and cell survival. *Proc. Natl. Acad. Sci. USA* 97: 4666–4671
- Tanaka S, Saito K and Reed JC (1993) Structure-function analysis of the Bcl-2 oncoprotein. Addition of a heterologous transmembrane domain to portions of the Bcl-2 beta protein restores function as a regulator of cell survival. *J. Biol. Chem.* 268: 10920–10926
- Kelekar A and Thompson CB (1998) Bcl-2-family proteins: the role of the BH3 domain in apoptosis. *Trends Cell. Biol.* 8: 324–330
- Wang K, Yin XM, Chao DT, Milliman CL and Korsmeyer SJ (1996) BID: a novel BH3 domain-only death agonist. *Genes Dev.* 10: 2859–2869
- Yang E, Zha J, Jockel J, Boise LH, Thompson CB and Korsmeyer SJ (1995) Bad, a heterodimeric partner for Bcl-X_L and Bcl-2, displaces Bax and promotes cell death. *Cell* 80: 285–291
- O'Connor L, Strasser A, O'Reilly LA, Hausmann G, Adams JM, Cory S and Huang DC (1998) Bim: a novel member of the Bcl-2 family that promotes apoptosis. *EMBO J.* 17: 384–395
- Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G and Thompson CB (1993) BCL-X, a BCL-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* 74: 597–608
- Sattler M, Liang H, Nettekheim D, Meadows RP, Harlan JE, Eberstadt M, Yoon HS, Shuker SB, Chang BS, Minn AJ, Thompson CB and Fesik SW (1997) Structure of Bcl-x_L-Bak peptide complex: recognition between regulators of apoptosis. *Science* 275: 983–986
- Minn AJ, Kettlun CS, Liang H, Kelekar A, Vander Heiden MG, Chang BS, Fesik SW, Fill M and Thompson CB (1999) Bcl-x_L regulates apoptosis by heterodimerization-dependent and -independent mechanisms. *EMBO J.* 18: 632–643
- Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettekheim D, Chang BS, Thompson CB, Wong SL, Ng SL and Fesik SW (1996) X-ray and NMR structure of human Bcl-x_L, an inhibitor of programmed cell death. *Nature* 381: 335–341
- Minn AJ, Velez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M and Thompson CB (1997) Bcl-x_L forms an ion channel in synthetic lipid membranes. *Nature* 385: 353–357
- Schlesinger PH, Gross A, Yin XM, Yamamoto K, Saito M, Waksman G and Korsmeyer SJ (1997) Comparison of the ion channel characteristics of proapoptotic Bax and antiapoptotic Bcl-2. *Proc. Natl. Acad. Sci. USA* 94: 11357–11362
- Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M and Reed JC (1997) Channel formation by antiapoptotic protein Bcl-2. *Proc. Natl. Acad. Sci. USA* 94: 5113–5118
- Antonsson B, Conti F, Ciavatta A, Montessuit S, Lewis S, Martinou I, Bernasconi L, Bernard A, Mermod JJ, Mazzei G, Maundrell K, Gambale F, Sadoul R and Martinou JC (1997) Inhibition of Bax channel-forming activity by Bcl-2. *Science* 277: 370–372
- Chou JJ, Li H, Salvesen GS, Yuan J and Wagner G (1999) Solution structure of BID, an intracellular amplifier of apoptotic signaling. *Cell* 96: 615–624
- 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
- Schendel SL, Azimov R, Pawlowski K, Godzik A, Kagan BL and Reed JC (1999) Ion channel activity of the BH3 only Bcl-2 family member, BID. *J. Biol. Chem.* 274: 21932–21936
- Kluck RM, Esposti MD, Perkins G, Renken C, Kuwana T, Bossy-Wetzel E, Goldberg M, Allen T, Barber MJ, Green DR and Newmeyer DD (1999) The proapoptotic proteins, Bid and Bax, cause a limited permeabilization of the mitochondrial outer membrane that is enhanced by cytosol. *J. Cell. Biol.* 147: 809–822

39. Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT and Thompson CB (1997) Bcl-x_L regulates the membrane potential and volume homeostasis of mitochondria. *Cell* 91: 627–637
40. Funk RH, Nagel F, Wonka F, Krinke HE, Golfert F and Hofer A (1999) Effects of heat shock on the functional morphology of cell organelles observed by video-enhanced microscopy. *Anat. Rec.* 255: 458–464
41. Welch WJ and Suhan JP (1985) Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. *J. Cell. Biol.* 101: 1198–1211
42. Halestrap AP (1989) The regulation of the matrix volume of mammalian mitochondria in vivo and in vitro and its role in the control of mitochondrial metabolism. *Biochim. Biophys. Acta* 973: 355–382
43. Rutka JT, Giblin JR, Berens ME, Bar-Shiva E, Tokuda K, McCulloch JR, Rosenblum ML, Eessalu TE, Aggarwal BB and Bodell WJ (1988) The effects of human recombinant tumor necrosis factor on glioma-derived cell lines: cellular proliferation, cytotoxicity, morphological and radioreceptor studies. *Int. J. Cancer* 41: 573–582
44. Hefner RR and Barron SA (1978) The early effects of ischemia upon skeletal muscle mitochondria. *J. Neurol. Sci.* 38: 295–315
45. Hackenbrock CR (1966) Ultrastructural bases for metabolically linked mechanical activity in mitochondria. I. Reversible ultrastructural changes with change in metabolic steady state in isolated liver mitochondria. *J. Cell. Biol.* 30: 269–297
46. Liu MY and Colombini M (1992) Regulation of mitochondrial respiration by controlling the permeability of the outer membrane through the mitochondrial channel, VDAC. *Biochim. Biophys. Acta* 1098: 255–260
47. Dang CV and Semenza GL (1999) Oncogenic alterations of metabolism. *Trends Biochem. Sci.* 24: 68–72
48. Vander Heiden MG, Chandel NS, Schumacker PT and Thompson CB (1999) Bcl-x_L prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange. *Mol. Cell* 3: 159–167
49. Kwong J, Choi HL, Huang Y and Chan FL (1999) Ultrastructural and biochemical observations on the early changes in apoptotic epithelial cells of the rat prostate induced by castration. *Cell Tissue Res.* 298: 123–136
50. Vander Heiden MG and Thompson CB (1999) Bcl-2 proteins: regulators of apoptosis or of mitochondrial homeostasis? *Nat. Cell Biol.* 1: E209–E216
51. Saks VA, Chernousova GB, Gukovsky DE, Smirnov VN and Chazov EI (1975) Studies of energy transport in heart cells. Mitochondrial isoenzyme of creatine phosphokinase: kinetic properties and regulatory action of Mg²⁺ ions. *Eur. J. Biochem.* 57: 273–290
52. Kennedy SG, Kandel ES, Cross TK and Hay N (1999) Akt/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. *Mol. Cell. Biol.* 19: 5800–5810
53. Gross A, Pilcher K, Blachly-Dyson E, Basso E, Jockel J, Bassik MC, Korsmeyer SJ and Forte M (2000) Biochemical and genetic analysis of the mitochondrial response of yeast to Bax and Bcl-x_L. *Mol. Cell. Biol.* 20: 3125–3136
54. Kane DJ, Sarafian TA, Anton R, Hahn H, Gralla EB, Valentine JS, Ord T and Bredesen DE (1993) Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* 262: 1274–1277
55. Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, Susin SA, Petit PX, Mignotte B and Kroemer G (1995) Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. *J. Exp. Med.* 182: 367–377
56. Hockenbery DM, Oltvai ZN, Yin XM, Millman CL and Korsmeyer SJ (1993) Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75: 241–251
57. Rostovtseva T and Colombini M (1996) ATP flux is controlled by a voltage-gated channel from the mitochondrial outer membrane. *J. Biol. Chem.* 271: 28006–28008
58. Vander Heiden MG, Li XX, Gottlieb E, Hill RB, Thompson CB and Colombini M. Bcl-x_L promotes the open configuration of VDAC and metabolite passage through the outer mitochondrial membrane. Submitted
59. Khaled AR, Kim K, Hofmeister R, Muegge K and Durum SK (1999) Withdrawal of IL-7 induces Bax translocation from cytosol to mitochondria through a rise in intracellular pH. *Proc. Natl. Acad. Sci. USA* 96: 14476–14481
60. Harris MH, Vander Heiden MG, Kron SJ and Thompson CB (2000) Role of oxidative phosphorylation in Bax toxicity. *Mol. Cell Biol.* 20: 3590–3596
61. Mignotte B and Vayssières JL (1998) Mitochondria and apoptosis. *Eur. J. Biochem.* 252: 1–15
62. Banki K, Hutter E, Gonchoroff NJ and Perl A (1999) Elevation of mitochondrial transmembrane potential and reactive oxygen intermediate levels are early events and occur independently from activation of caspases in Fas signaling. *J. Immunol.* 162: 1466–1479
63. Gottlieb E, Vander Heiden M and Thompson C (2000) Bcl-x_L prevents the initial decrease in mitochondrial membrane potential and subsequent reactive oxygen species production during tumor necrosis factor alpha-induced apoptosis. *Mol. Cell. Biol.* 20: 5680–5689
64. Zamzami N, Susin SA, Marchetti P, Hirsch T, Gomez-Monterrey I, Castedo M and Kroemer G (1996) Mitochondrial control of nuclear apoptosis. *J. Exp. Med.* 183: 1533–1544
65. Kroemer G, Dallaporta B and Resche-Rigon M (1998) The mitochondrial death/life regulator in apoptosis and necrosis. *Annu. Rev. Physiol.* 60: 619–642
66. Zoratti M and Szabo I (1995) The mitochondrial permeability transition. *Biochim. Biophys. Acta* 1241: 139–176
67. Crompton M (1999) The mitochondrial permeability transition pore and its role in cell death. *Biochem. J.* 341: 233–249
68. Brdiczka D, Beutner G, Ruck A, Dolder M and Wallimann T (1998) The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition. *Biofactors* 8: 235–242
69. Zamzami N, Marchetti P, Castedo M, Hirsch T, Susin SA, Masse B and Kroemer G (1996) Inhibitors of permeability transition interfere with the disruption of the mitochondrial transmembrane potential during apoptosis. *FEBS Lett.* 384: 53–57
70. Bradham CA, Qian T, Streetz K, Trautwein C, Brenner DA and Lemasters JJ (1998) The mitochondrial permeability transition is required for tumor necrosis factor alpha-mediated apoptosis and cytochrome c release. *Mol. Cell. Biol.* 18: 6353–6364
71. Matsuyama S, Llopis J, Deveraux QL, Tsien RY and Reed JC (2000) Changes in intramitochondrial and cytosolic pH: early events that modulate caspase activation during apoptosis. *Nat. Cell Biol.* 2: 318–325
72. Shimizu S, Ide T, Yanagida T and Tsujimoto Y (2000) Electrophysiological study of a novel large pore formed by Bax and the voltage-dependent anion channel that is permeable to cytochrome c. *J. Biol. Chem.* 275: 12321–12325
73. Shimizu S, Narita M and Tsujimoto Y (1999) Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC [see comments]. *Nature* 399: 483–487
74. Schein SJ, Colombini M and Finkelstein A (1976) Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from paramecium mitochondria. *J. Membr. Biol.* 30: 99–120
75. Colombini M (1989) Voltage gating in the mitochondrial channel, VDAC. *J. Membr. Biol.* 111: 103–111
76. Shimizu S, Konishi A, Kodama T and Tsujimoto Y (2000) BH4 domain of antiapoptotic Bcl-2 family members closes voltage-dependent anion channel and inhibits apoptotic mitochondrial changes and cell death. *Proc. Natl. Acad. Sci. USA* 97: 3100–3105
77. Cheng EH, Kirsch DG, Clem RJ, Ravi R, Kastan MB, Bedi A, Ueno K and Hardwick JM (1997) Conversion of Bcl-2 to a Bax-like death effector by caspases. *Science* 278: 1966–1968
78. Kirsch DG, Doseff A, Chau BN, Lim DS, de Souza-Pinto NC, Hansford R, Kastan MB, Lazebnik YA and Hardwick JM (1999) Caspase-3-dependent cleavage of Bcl-2 promotes release of cytochrome c. *J. Biol. Chem.* 274: 21155–21161
79. Jurgensmeier JM, Xie Z, Deveraux Q, Ellerby L, Bredesen D and Reed JC (1998) Bax directly induces release of cytochrome c from isolated mitochondria. *Proc. Natl. Acad. Sci. USA* 95: 4997–5002
80. Hsu YT, Wolter KG and Youle RJ (1997) Cytosol-to-membrane redistribution of Bax and Bcl-X_L during apoptosis. *Proc. Natl. Acad. Sci. USA* 94: 3668–3672
81. Wolter KG, Hsu YT, Smith CL, Nechushtan A, Xi XG and Youle RJ (1997) Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell. Biol.* 139: 1281–1292
82. 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
83. Gross A, Jockel J, Wei MC and Korsmeyer SJ (1998) Enforced dimerization of Bax results in its translocation, mitochondrial dysfunction and apoptosis. *EMBO J.* 17: 3878–3885

84. Desagher S, Osen-Sand A, Nichols A, Eskes R, Montessuit S, Lauper S, Maundrell K, Antonsson B and Martinou JC (1999) Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. *J. Cell. Biol.* 144: 891–901
85. 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
86. 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
87. Antonsson B, Montessuit S, Lauper S, 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 Pt2: 271–278
88. Puthalakath H, Huang DC, O'Reilly LA, King SM and Strasser A (1999) The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol. Cell.* 3: 287–296
89. Inohara N, Ding L, Chen S and Nunez G (1997) Harakiri, a novel regulator of cell death, encodes a protein that activates apoptosis and interacts selectively with survival-promoting proteins Bcl-2 and Bcl-x_L. *EMBO J.* 16: 1686–1694
90. Han J, Sabbatini P and White E (1996) Induction of apoptosis by human Nbk/Bik, a BH3-containing protein that interacts with E1B 19K. *Mol. Cell. Biol.* 16: 5857–5864
91. Xiang J, Chao DT and Korsmeyer SJ (1996) BAX-induced cell death may not require interleukin 1 beta-converting enzyme-like proteases. *Proc. Natl. Acad. Sci. USA* 93: 14559–14563
92. Sato T, Hanada M, Bodrug S, Irie S, Iwama N, Boise LH, Thompson CB, Golemis E, Fong L, Wang HG and Reed SC (1994) Interactions among members of the Bcl-2 protein family analyzed with a yeast two-hybrid system [published erratum appears in *Proc. Natl. Acad. Sci. USA* 1995 Feb 28;92(5):2016]. *Proc. Natl. Acad. Sci. USA* 91: 9238–9242