Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases

David G Breckenridge and Ding Xue[1]

Mitochondria play an important role in the integration and transmission of cell death signals, activating caspases and other cell death execution events by releasing apoptogenic proteins from the intermembrane space. The BCL-2 family of proteins localize (or can be targeted) to mitochondria and regulate the permeability of the mitochondrial outer membrane to these apoptotic factors. Recent evidence suggests that multiple mechanisms may regulate the release of mitochondrial factors, some of which depend on the action of caspases.

Addresses
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309, USA
[1]e-mail: ding.xue@colorado.edu

Current Opinion in Cell Biology 2004, **16**:647–652

This review comes from a themed issue on
Cell division, growth and death
Edited by Steve Reed and Joel Rothman

Available online 13th October 2004

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2004.09.009

Abbreviations

AIF	apoptosis-inducing factor
BH	BCL-2 homology
cyt.c	cytochrome *c*
EndoG	endonuclease G
ER	endoplasmic reticulum
IAP	inhibitor of apoptosis
IMM	inner mitochondrial membrane
IMS	intermembrane space
MMP	mitochondrial membrane permeabilization
mtPTP	mitochondrial permeability transition pore
OMM	outer mitochondrial membrane
ROS	reactive oxygen species
tBID	truncated BID

Introduction

In mammals, diverse death stimuli induce apoptosis by activating the caspase family of cysteine proteases and various cell disassembly processes. In many instances this task is mediated by mitochondria, which sense incoming apoptotic signals and respond to them by releasing apoptogenic factors from the mitochondrial intermembrane space (IMS) into the cytosol and the nucleus, where they trigger caspase activation and other cell death events. For instance, mitochondrial release of cytochrome *c* (cyt.c) triggers the assembly of a cytosolic caspase activation complex, the apoptosome, whereas release of Smac/DIABLO and Htra2/Omi inactivates the inhibitors of apoptosis (IAPs), a family of caspase inhibitors, facilitating the activation of caspases [1]. Mitochondria also release apoptosis-inducing factor (AIF) and endonuclease G (EndoG), which enter the nucleus and cooperate to degrade nuclear DNA [2–4,5•]. The precise mechanisms by which the permeability of the outer mitochondrial membrane (OMM) becomes compromised during apoptosis (hereafter referred to as mitochondrial membrane permeabilization, MMP) and by which the apoptogenic factors are released have remained elusive, although it is clear that these processes are governed by the BCL-2 family of proteins and in some cases by caspases themselves. The role of mitochondria during apoptosis has been investigated thoroughly and is reviewed in detail elsewhere (for example see [6–8]). Here, we focus on recent findings that contribute to our understanding of the regulation of MMP by BCL-2 proteins and caspases.

BCL-2 family proteins and mitochondria

BCL-2 family proteins can generally be subdivided into three classes on the basis of their functions and the number of BCL-2 homology (BH) domains present: the anti-apoptotic members such as BCL-2 and BCL-xL that have four BH domains (BH1 to BH4), the pro-apoptotic members such as BAK and BAX that possess three BH domains (BH1 to BH3), and the 'BH3-only' pro-apoptotic members such as Bid and Bim that share homology only within the BH3 domain [9]. Proteins in these three classes are capable of forming either homo-oligomers or heterodimers with one another and appear to play distinct roles in governing MMP. BAX and BAK, each of which can form homo-oligomers in the OMM in response to apoptotic signals, constitute a partially redundant, but requisite, gateway for OMM permeabilization because cells doubly deficient for these two proteins are resistant to cyt.c release and apoptosis induced by multiple apoptotic stimuli [10,11]. By contrast, cells deficient for individual BH3-only proteins are resistant to cyt.c release or cell death induced by selective cell death signals [12–15]. BH3-only proteins such as BAD, BIM and BID appear to function upstream of BAX and BAK, because ectopic expression of these proteins could not induce cyt.c release and apoptosis in cells lacking both BAX and BAK [10,11,16]. In addition, overexpression of BCL-2 or BCL-xL can block MMP induced by ectopically expressed BH3-only proteins or by BAX and/or BAK [17]. These observations suggest that the interplay of

different classes of BCL-2 family proteins is crucial in determining the ultimate mitochondrial response. It is now widely accepted that distinct apoptotic signals first converge upon different BH3-only proteins, which upon activation deliver the death signals to mitochondria by engaging BAX/BAK or BCL-2/BCL-xL. BH3-only proteins are typically activated by either post-translational modifications, such as caspase-8-mediated cleavage of BID into an activated, truncated BID (tBID), or by transcriptional upregulation (reviewed in [18]).

In healthy cells, BAK is held in an inactive, monomeric state in the OMM through its association with VDAC-2 [19], whereas BAX may lay dormant in the cytosol through interactions with several proteins, including Ku-70 [20], 14-3-3 [21] and the humanin peptide [22]. Many apoptotic signals can trigger BH3-only-protein-dependent translocation of Bax, followed by its insertion into the OMM [23] and the formation of BAK or BAX homo-oligomers [24,25,26••], which are likely exit conduits in the OMM for apoptotic IMS proteins (see below). Several models have been proposed for how BCL-2 anti-apoptotic proteins antagonize the functions of BAX, BAK and BH3-only proteins. Cheng *et al.*, reported that the ability of BCL-2 or BCL-xL to inhibit apoptosis induced by BID, BIM or BAD expression correlates with their ability to bind the BH3-only proteins but not BAX/BAK [16]. This observation suggests that BCL-2 anti-apoptotic proteins may act by sequestering active BH3-only proteins away from BAX/BAK. However, increased association between BCL-2 and BAK is observed following binding of tBID to BAK [19,27], suggesting that BCL-2 might also play a direct role in regulating BAK or BAX oligomerization. In addition, the membrane topology of BCL-2 alters during apoptosis, or following treatment of isolated mitochondria with BIM, reflecting a potential BCL-2 conformational change in response to an activated BAK/BAX complex [28]. Thus, BCL-2 anti-apoptotic proteins may function both by sequestering active BH3-only proteins and by restricting BAX/BAK oligomerization, thereby setting an activation barrier for the induction of apoptosis and limiting inappropriate cell death. During apoptosis, the anti-apoptotic activity of BCL-2 is probably overcome by simultaneous activation of distinct classes of BH3-only proteins, some of which bind to and inactivate BCL-2, setting 'activator' BH3-only proteins free to activate BAX/BAK [29•].

How do BAX and BAK mediate permeabilization of the OMM? Clues come from the structures of BCL-2, BCL-xL and BAX, all of which reveal a striking similarity to those of the pore-forming domains of bacterial toxins [30–32]. A simple model for MMP, therefore, is that BH3-only proteins induce an allosteric conformational change in BAX/BAK, triggering their oligomerization into large pores in the OMM. Indeed, Newmeyer and colleagues found that tBID and BAX (or oligomerized BAX alone) could form supramolecular openings in reconstituted liposomes, allowing the passage of two-megadalton dextrans [26••]. Interestingly, the formation of these membrane openings is absolutely dependent on the presence of cardiolipin, a lipid enriched in the inner mitochondrial membrane (IMM) and at IMM and OMM contact sites [33]. tBID and BCL-2 have been shown to cluster at such contact points [34–36], which may be local sites of action for BCL-2 proteins. While the biophysical properties of a cardiolipin-enriched environment might be important for BAX/BAK to promote the local membrane curvature needed for the formation of a lipidic pore [26••], it remains unclear whether BAX and BAK act at contact sites or non-contact sites to permeabilize the OMM.

Several lines of evidence suggest that regulation of MMP by BCL-2 proteins entails more than BAX/BAK punching holes in the OMM to allow IMS proteins to leak out. First, the release profiles of different IMS proteins vary in their timings and dependence on other factors such as caspases (see below), suggesting that there are upstream checkpoints that must be met before a given IMS protein is released. Second, mitochondria appear to undergo several changes in membrane structure and morphology before the release of cyt.c, including mitochondrial fission [37], IMM cristae remodeling [36,38•] and lipid peroxidation [39]; all these changes appear to affect cyt.c release. Third, numerous studies indicate that transient openings in the mitochondrial permeability transition pore (mtPTP), a large, high-conductance multi-protein complex that spans the IMM and OMM, are important for the release of cyt.c [38•,40,41]. And fourth, BCL-2 family proteins such as BAX, BAK and BCL-2 also localize to the endoplasmic reticulum (ER) and can affect ER Ca^{2+} homeostasis and Ca^{2+} uptake by mitochondria, which may be important for mtPTP opening and IMS protein release in some instances [42–45]. Some of these phenomena may be explained by interactions that have been observed between BCL-2 family proteins and components of the mtPTP [46,47] or the mitochondrial fission machinery [48]. It is also possible that BAX and BAK cooperate with the mtPTP to form a channel in the OMM that is inhibited by BCL-2 (reviewed in [49]). Thus, BCL-2 family proteins may activate numerous processes in the mitochondria and ER during apoptosis, including reorganization of apoptogenic proteins within the IMS before their ultimate passage across the OMM.

Caspase activation and mitochondrial factor release, which comes first?

Although it is clear that MMP is involved in the activation of caspases, there has been considerable debate over whether MMP can occur independently of caspase activities. In the case of extrinsic cytokine signaling through cell surface death receptors, it is clear that initiator caspases such as caspase-8 can induce MMP by cleaving BID into tBID [17,50,51]; tBID then amplifies weak

initiator caspase signals by evoking the release of cyt.c, Smac/Diablo and Htra2/Omi to activate the apoptosome and to relieve IAP inhibition of caspases [52]. In the case of intrinsic death signals such as genotoxic stresses, the kinetics of cyt.c release do not seem to be affected by caspase inhibitors, suggesting that cyt.c release can also occur independently of caspases [40,53]. However, proof of a truly caspase-independent MMP pathway is hard to come by given that some caspases may be insensitive to commonly used caspase inhibitors [54]. Caspase-2, for example, is zVAD-FMK-insensitive and can directly evoke the release of cyt.c and SMAC from isolated mitochondria [55–57]. Indeed, siRNA knock-down of caspase-2 blocks cyt.c and SMAC release from mitochondria following genotoxic stresses in some human tumor cell lines [58]. Interestingly, caspase-2 may not require its enzymatic activity to cause MMP [57], suggesting alternative regulatory functions for caspases and calling into question results obtained from caspase inhibitors. However, genetic elimination of caspase-2 in mice does not result in a significant defect in developmental cell deaths [59]. Thus, in many cellular contexts, caspase-2 might play a non-essential, or perhaps redundant, role in mediating the release of mitochondrial apoptogenic factors.

Different IMS proteins may have different modes of escape from mitochondria, some being caspase-dependent and others not. For example, it has been reported that zVAD-fmk could inhibit the release of SMAC [60], AIF and EndoG [61•], but not of cyt.c, from mitochondria following intrinsic death insults. Furthermore, in [61•] it was shown that tBID or oligomerized BAX could release cyt.c, but not AIF and EndoG, from isolated mitochondria. These observations are consistent with studies in *Caenorhabditis elegans* showing that release of WAH-1, a worm AIF orthologue, from mitochondria by the BH3-only protein EGL-1 is strongly inhibited by a loss-of-function mutation in the CED-3 caspase [5•]. It is not clear whether the limited release of WAH-1 observed in the *ced-3* mutant represents a *bona fide* caspase-independent event or is caused by other *C. elegans* caspases [62]. Nonetheless, these findings, and the observation that AIF and EndoG appear to be more tightly associated with the IMM than cyt.c [61•], suggest that caspase-mediated proteolysis of certain cytosolic or mitochondrial components may be crucial to promote the transport of these IMS proteins across the OMM (see below).

How might caspases exert their effects on mitochondria? One mechanism is through the cleavage and activation of the BH3-only proteins such as BID [50,51] and BAD [63], or cleavage and reversal of the anti-apoptotic functions of BCL-2 [64] and BCL-xL [65]. Recent reports by Green and colleagues revealed that caspases can cause mitochondrial respiratory dysfunction following limited permeabilization of the OMM by BAX/BAK [66,67••]. Caspase-3 cleaves the 75-kD subunit of complex I of the electron transport chain, and probably another component of complex II, resulting in disruption of electron flow from complex I and II, loss of mitochondrial transmembrane potential ($\Delta\psi m$), an increased production of reactive oxygen species (ROS), and disruption of mitochondrial morphology; all of these are events that normally occur during apoptosis but are prevented by zVAD-fmk [66]. Interestingly, all of these events are attenuated in cells expressing a caspase-resistant p75 mutant, although cyt.c release occurs normally. These observations suggest a likely sequential process for activating MMP involving an initial release of cyt.c without disruption of the respiratory processes. This would ensure a sufficient supply of ATP for various apoptotic processes while caspases are being activated in the cytosol [40]. By this model, once activated, caspases would enter mitochondria through the partially permeabilized OMM and mount a catastrophic attack, crippling energy production and ensuring the demise of the cell. In addition, the resulting production of ROS, concomitant lipid peroxidation, gross organelle swelling and morphological disruption [67••] might then further facilitate the dissociation and release of AIF and EndoG from the IMM [61•].

Conclusions

Genetic and biochemical studies have demonstrated that BCL-2 family proteins are central to the regulation of MMP. A major challenge now is to investigate how these proteins act on mitochondria to influence MMP. At present, it seems reasonable to conclude that both caspase-dependent and -independent pathways of MMP exist; which one is used probably depends on the cellular context and death stimuli. In some instances, MMP may occur in a multi-step process in which cyt.c, SMAC and HtrA2/Omi are released first, allowing caspase activation, followed by caspase-dependent release of AIF and EndoG [61•]. This may ensure that complete nuclear DNA degradation occurs only after caspases are activated and the respiratory potential of the cell is lost. Notably, however, reports of a BID cleavage product, jBID, that induces Smac release from mitochondria in the absence of cyt.c release [68], and the finding that AIF release can occur independently of caspases in some cases [69], suggest that the picture maybe more complicated than anticipated. It will be important to determine the molecular basis for these different release modes of apoptogenic factors from mitochondria. Finally, in light of the fact that the mitochondrion also seems to play an important role during cell death in nematodes [4,5•,70] and yeast (G Kroemer, personal communication), these model organisms may prove powerful for genetically delineating pathways that regulate the release of mitochondrial apoptogenic factors, a task that could be difficult to pursue in higher organisms.

Acknowledgements

Due to space limit, we apologize for not being able to cite all the references. David Breckenridge is a fellow of the Jane Coffins Fund for

Medical Research. Research in Ding Xue's laboratory is supported by a Searle Scholar award, a Burroughs Wellcome Fund Career Award, and grants from NIH.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jiang X, Wang X: **Cytochrome-*c*-mediated apoptosis**. *Annu Rev Biochem* 2004, **73**:87-106.

2. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M *et al.*: **Molecular characterization of mitochondrial apoptosis-inducing factor**. *Nature* 1999, **397**:441-446.

3. Li LY, Luo X, Wang X: **Endonuclease G is an apoptotic DNase when released from mitochondria**. *Nature* 2001, **412**:95-99.

4. Parrish J, Li L, Klotz K, Ledwich D, Wang X, Xue D: **Mitochondrial endonuclease G is important for apoptosis in *C. elegans***. *Nature* 2001, **412**:90-94.

5. • Wang X, Yang C, Chai J, Shi Y, Xue D: **Mechanisms of AIF-mediated apoptotic DNA degradation in *Caenorhabditis elegans***. *Science* 2002, **298**:1587-1592.

This article reveals that the worm orthologs of AIF and EndoG, WAH-1 and CPS-6, respectively, cooperate to degrade nuclear DNA during cell death in *C. elegans*. In addition, it is shown that the BH3-only protein EGL-1 could induce the release of WAH-1 from mitochondria in a CED-3-caspase-dependent manner. This study and [61•] suggest an evolutionarily conserved mechanism for AIF release from mitochondria.

6. Danial NN, Korsmeyer SJ: **Cell death: critical control points**. *Cell* 2004, **116**:205-219.

7. Wang X: **The expanding role of mitochondria in apoptosis**. *Genes Dev* 2001, **15**:2922-2933.

8. Newmeyer DD, Ferguson-Miller S: **Mitochondria: releasing power for life and unleashing the machineries of death**. *Cell* 2003, **112**:481-490.

9. Gross A, McDonnell JM, Korsmeyer SJ: **BCL-2 family members and the mitochondria in apoptosis**. *Genes Dev* 1999, **13**:1899-1911.

10. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB, Korsmeyer SJ: **Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death**. *Science* 2001, **292**:727-730.

11. Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB: **BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak**. *Genes Dev* 2001, **15**:1481-1486.

12. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, Adams JM, Strasser A: **Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity**. *Science* 1999, **286**:1735-1738.

13. Coultas L, Bouillet P, Stanley EG, Brodnicki TC, Adams JM, Strasser A: **Proapoptotic BH3-only Bcl-2 family member Bik/Blk/Nbk is expressed in hemopoietic and endothelial cells but is redundant for their programmed death**. *Mol Cell Biol* 2004, **24**:1570-1581.

14. Villunger A, Michalak EM, Coultas L, Mullauer F, Bock G, Ausserlechner MJ, Adams JM, Strasser A: **p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa**. *Science* 2003, **302**:1036-1038.

15. Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, Roth KA, Korsmeyer SJ: **Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis**. *Nature* 1999, **400**:886-891.

16. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ: **BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis**. *Mol Cell* 2001, **8**:705-711.

17. Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, Erdjument-Bromage H, Tempst P, Korsmeyer SJ: **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* 1999, **274**:1156-1163.

18. Puthalakath H, Strasser A: **Keeping killers on a tight leash: transcriptional and post-translational control of the pro-apoptotic activity of BH3-only proteins**. *Cell Death Differ* 2002, **9**:505-512.

19. Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ: **VDAC2 inhibits BAK activation and mitochondrial apoptosis**. *Science* 2003, **301**:513-517.

20. Sawada M, Sun W, Hayes P, Leskov K, Boothman DA, Matsuyama S: **Ku70 suppresses the apoptotic translocation of Bax to mitochondria**. *Nat Cell Biol* 2003, **5**:320-329.

21. Tsuruta F, Sunayama J, Mori Y, Hattori S, Shimizu S, Tsujimoto Y, Yoshioka K, Masuyama N, Gotoh Y: **JNK promotes Bax translocation to mitochondria through phosphorylation of 14-3-3 proteins**. *EMBO J* 2004, **23**:1889-1899.

22. Guo B, Zhai D, Cabezas E, Welsh K, Nouraini S, Satterthwait AC, Reed JC: **Humanin peptide suppresses apoptosis by interfering with Bax activation**. *Nature* 2003, **423**:456-461.

23. Ruffolo SC, Breckenridge DG, Nguyen M, Goping IS, Gross A, Korsmeyer SJ, Li H, Yuan J, Shore GC: **BID-dependent and BID-independent pathways for BAX insertion into mitochondria**. *Cell Death Differ* 2000, **7**:1101-1108.

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

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

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

Using an *in vitro* vesicular reconstitution method, the authors find that tBID can activate BAX to form large openings in vesicles derived from the OMM or in defined liposomes. This is the first indication that BAX homo-oligomers may act on the OMM alone, independent of other mitochondrial proteins, to allow the release of large molecules.

27. Ruffolo SC, Shore GC: **BCL-2 selectively interacts with the BID-induced open conformer of BAK, inhibiting BAK auto-oligomerization**. *J Biol Chem* 2003, **278**:25039-25045.

28. Kim PK, Annis MG, Dlugosz PJ, Leber B, Andrews DW: **During apoptosis bcl-2 changes membrane topology at both the endoplasmic reticulum and mitochondria**. *Mol Cell* 2004, **14**:523-529.

29. • Letai A, Bassik M, Walensky L, Sorcinelli M, Weiler S, Korsmeyer S: **Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics**. *Cancer Cell* 2002, **2**:183-192.

By analyzing the ability of BH3 domain peptides derived from different BH3-only proteins to induce oligomerization of BAK/BAK, and by comparing the binding affinity of the BH3 peptides for BAX/BAK versus BCL-2, the authors provide evidence for the existence of two functional classes of BH3-only proteins: 'sensitizer' BH3s that bind BCL-2 anti-apoptotic proteins and prevent their sequestration of 'activator' BH3s, which activate BAX/BAK. This is the first report to address differential functions for the BH3-only proteins.

30. Suzuki M, Youle RJ, Tjandra N: **Structure of Bax: coregulation of dimer formation and intracellular localization**. *Cell* 2000, **103**:645-654.

31. Sattler M, Liang H, Nettesheim D, Meadows RP, Harlan JE, Eberstadt M, Yoon HS, Shuker SB, Chang BS, Minn AJ *et al.*: **Structure of Bcl-xL-Bak peptide complex:**

recognition between regulators of apoptosis. *Science* 1997, **275**:983-986.

32. Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettesheim D, Chang BS, Thompson CB, Wong SL *et al.*: **X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death**. *Nature* 1996, **381**:335-341.

33. Lutter M, Fang M, Luo X, Nishijima M, Xie X, Wang X: **Cardiolipin provides specificity for targeting of tBid to mitochondria**. *Nat Cell Biol* 2000, **2**:754-761.

34. Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W, Reed JC: **Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes**. *Cancer Res* 1993, **53**:4701-4714.

35. Lutter M, Perkins GA, Wang X: **The pro-apoptotic Bcl-2 family member tBid localizes to mitochondrial contact sites**. *BMC Cell Biol* 2001, **2**:22.

36. Kim TH, Zhao Y, Ding WX, Shin JN, He X, Seo YW, Chen J, Rabinowich H, Amoscato AA, Yin XM: **Bid-cardiolipin interaction at mitochondrial contact site contributes to mitochondrial cristae reorganization and cytochrome C release**. *Mol Biol Cell* 2004, **15**:3061-3072.

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

38. • Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, Korsmeyer SJ: **A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis**. *Dev Cell* 2002, **2**:55-67.

The authors report a distinct pathway leading to mitochondrial cristae remodeling during apoptosis and suggest that such IMM reorganization leads to mobilization of cyt.c stores out of tight cristae junctions and into the IMS, allowing the passage and complete release of cyt.c across the OMM.

39. Ott M, Robertson JD, Gogvadze V, Zhivotovsky B, Orrenius S: **Cytochrome c release from mitochondria proceeds by a two-step process**. *Proc Natl Acad Sci USA* 2002, **99**:1259-1263.

40. Goldstein JC, Waterhouse NJ, Juin P, Evan GI, Green DR: **The coordinate release of cytochrome c during apoptosis is rapid, complete and kinetically invariant**. *Nat Cell Biol* 2000, **2**:156-162.

41. Zamzami N, Kroemer G: **The mitochondrion in apoptosis: how Pandora's box opens**. *Nat Rev Mol Cell Biol* 2001, **2**:67-71.

42. Nutt LK, Chandra J, Pataer A, Fang B, Roth JA, Swisher SG, O'Neil RG, McConkey DJ: **Bax-mediated Ca^{2+} mobilization promotes cytochrome c release during apoptosis**. *J Biol Chem* 2002, **277**:20301-20308.

43. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, Korsmeyer SJ: **BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis**. *Science* 2003, **300**:135-139.

44. Zong WX, Li C, Hatzivassiliou G, Lindsten T, Yu QC, Yuan J, Thompson CB: **Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis**. *J Cell Biol* 2003, **162**:59-69.

45. Pinton P, Ferrari D, Magalhaes P, Schulze-Osthoff K, Di Virgilio F, Pozzan T, Rizzuto R: **Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells**. *J Cell Biol* 2000, **148**:857-862.

46. Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL, Prevost MC, Xie Z, Matsuyama S, Reed JC *et al.*: **Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis**. *Science* 1998, **281**:2027-2031.

47. Shimizu S, Narita M, Tsujimoto Y: **Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC**. *Nature* 1999, **399**:483-487.

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

49. Zamzami N, Kroemer G: **The mitochondrion in apoptosis: how Pandora's box opens**. *Nat Rev Mol Cell Biol* 2001, **2**:67-71.

50. Li H, Zhu H, Xu CJ, Yuan J: **Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis**. *Cell* 1998, **94**:491-501.

51. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X: **Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors**. *Cell* 1998, **94**:481-490.

52. Li S, Zhao Y, He X, Kim TH, Kuharsky DK, Rabinowich H, Chen J, Du C, Yin XM: **Relief of extrinsic pathway inhibition by the Bid-dependent mitochondrial release of Smac in Fas-mediated hepatocyte apoptosis**. *J Biol Chem* 2002, **277**:26912-26920.

53. Bossy-Wetzel E, Newmeyer DD, Green DR: **Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarisation**. *EMBO J* 1998, **17**:37-49.

54. Thornberry NA, Rano TA, Peterson EP, Rasper DM, Timkey T, Garcia-Calvo M, Houtzager VM, Nordstrom PA, Roy S, Vaillancourt JP *et al.*: **A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis**. *J Biol Chem* 1997, **272**:17907-17911.

55. Guo Y, Srinivasula SM, Druilhe A, Fernandes-Alnemri T, Alnemri ES: **Caspase-2 induces apoptosis by releasing proapoptotic proteins from mitochondria**. *J Biol Chem* 2002, **277**:13430-13437.

56. Robertson JD, Enoksson M, Suomela M, Zhivotovsky B, Orrenius S: **Caspase-2 acts upstream of mitochondria to promote cytochrome c release during etoposide-induced apoptosis**. *J Biol Chem* 2002, **277**:29803-29809.

57. Robertson JD, Gogvadze V, Kropotov A, Vakifahmetoglu H, Zhivotovsky B, Orrenius S: **Processed caspase-2 can induce mitochondria-mediated apoptosis independently of its enzymatic activity**. *EMBO Rep* 2004, **5**:643-648.

58. Lassus P, Opitz-Araya X, Lazebnik Y: **Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization**. *Science* 2002, **297**:1352-1354.

59. Bergeron L, Perez GI, Macdonald G, Shi L, Sun Y, Jurisicova A, Varmuza S, Latham KE, Flaws JA, Salter JC *et al.*: **Defects in regulation of apoptosis in caspase-2-deficient mice**. *Genes Dev* 1998, **12**:1304-1314.

60. Adrain C, Creagh EM, Martin SJ: **Apoptosis-associated release of Smac/DIABLO from mitochondria requires active caspases and is blocked by Bcl-2**. *EMBO J* 2001, **20**:6627-6636.

61. • Arnoult D, Gaume B, Karbowski M, Sharpe JC, Cecconi F, Youle RJ: **Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization**. *EMBO J* 2003, **22**:4385-4399.

By analyzing the release of cyt.c, Smac, Htra2, AIF and EndoG from mitochondria in intact cells and from isolated mitochondria *in vitro*, the authors demonstrate a distinct mode of release for AIF and EndoG that is dependent on caspase activities.

62. Shaham S: **Identification of multiple *Caenorhabditis elegans* caspases and their potential roles in proteolytic cascades**. *J Biol Chem* 1998, **273**:35109-35117.

63. Condorelli F, Salomoni P, Cotteret S, Cesi V, Srinivasula SM, Alnemri ES, Calabretta B: **Caspase cleavage enhances the apoptosis-inducing effects of BAD**. *Mol Cell Biol* 2001, **21**:3025-3036.

64. Cheng EH, Kirsch DG, Clem RJ, Ravi R, Kastan MB, Bedi A, Ueno K, Hardwick JM: **Conversion of Bcl-2 to a Bax-like death effector by caspases**. *Science* 1997, **278**:1966-1968.

65. Clem RJ, Cheng EH, Karp CL, Kirsch DG, Ueno K, Takahashi A, Kastan MB, Griffin DE, Earnshaw WC, Veliuona MA *et al.*:

Modulation of cell death by Bcl-XL through caspase interaction. *Proc Natl Acad Sci USA* 1998, **95**:554-559.

66. Ricci JE, Gottlieb RA, Green DR: **Caspase-mediated loss of mitochondrial function and generation of reactive oxygen species during apoptosis**. *J Cell Biol* 2003, **160**:65-75.

67. •• Ricci JE, Munoz-Pinedo C, Fitzgerald P, Bailly-Maitre B, Perkins GA, Yadava N, Scheffler IE, Ellisman MH, Green DR: **Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain**. *Cell* 2004, **117**:773-786.

This study identified the p75 subunit of complex I of the electron transport chain as a major caspase-3 substrate, and whose cleavage contributes to mitochondrial respiratory failure during apoptosis.

68. Deng Y, Ren X, Yang L, Lin Y, Wu X: **A JNK-dependent pathway is required for TNFα-induced apoptosis**. *Cell* 2003, **115**:61-70.

69. Penninger JM, Kroemer G: **Mitochondria, AIF and caspases — rivaling for cell death execution**. *Nat Cell Biol* 2003, **5**:97-99.

70. Bloss TA, Witze ES, Rothman JH: **Suppression of CED-3-independent apoptosis by mitochondrial βNAC in *Caenorhabditis elegans***. *Nature* 2003, **424**:1066-1071.