

Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article was published in an Elsevier journal. The attached copy is furnished to the author for non-commercial research and education use, including for instruction at the author's institution, sharing with colleagues and providing to institution administration.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>

# The daily job of night killers: alternative roles of the BCL-2 family in organelle physiology

Claudio Hetz<sup>1,2</sup> and Laurie Glimcher<sup>1,3</sup>

<sup>1</sup> Department of Immunology and Infectious diseases, Harvard School of Public Health, Boston, MA 02115, USA

<sup>2</sup> Department of Cellular and Molecular Biology, Institute of Biomedical Sciences, University of Chile and the FONDAF Center for Molecular Studies of the Cell (CEMC), Santiago, Chile

<sup>3</sup> Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

**Apoptosis is essential for maintenance of tissue homeostasis and its deregulation underlies many disease conditions. The BCL-2 family of proteins is a group of evolutionarily conserved regulators of cell death, comprising both anti- and pro-apoptotic members, which operate at the mitochondrial membrane to control caspase activation. Different BCL-2-related proteins are also located in multiprotein complexes at the endoplasmic reticulum (ER), which are involved in the control of diverse cellular processes, including calcium homeostasis, autophagy, the unfolded protein response and ER morphogenesis. Here, we describe the emerging concept that BCL-2-related proteins have alternative functions beyond apoptosis to control the essential functions of the cell.**

## Introduction

Complex signaling responses mediate adaptation to organelle stress or initiation of cell-death processes when a threshold of damage has been reached. Execution of apoptosis depends on the activation of caspases, a process regulated tightly by the BCL-2 family of proteins. The BCL-2 family of proteins is comprised of pro- and anti-apoptotic members that are defined by the presence of up to four conserved domains. Antiapoptotic BCL-2 family members display sequence homology in four  $\alpha$ -helical domains called BCL-2 homology (BH)1 to BH4 [1]. Proapoptotic BCL-2 members can be further subdivided into more highly conserved, 'multidomain' members displaying homology in the BH1, BH2 and BH3 (i.e. BAX and BAK) domains or the 'BH3-only' members (i.e. BIK, BIM, PUMA and NOXA), which contain a single domain crucial for the activation of apoptosis. Recently, a new subgroup of proapoptotic proteins, called BNip proteins, which have minimal sequence similarity in the BH3 domain, was identified (reviewed in [2]).

Each member of the BCL-2 family has distinct patterns of developmental expression, subcellular localization and differential responsiveness to specific death stimuli [3]. Some BH3-only proteins are thought to operate as sentinels

of cellular damage [1], where, in response to various death stimuli (i.e. oxidative stress, DNA damage or death-receptor engagement), they are activated either by transcriptional upregulation or through post-translational modifications. BH3-only proteins then promote the activation of the core proapoptotic components BAX and/or BAK [4], resulting in mitochondrial-membrane permeabilization [1]. Released mitochondrial proteins, such as cytochrome *c*, then trigger caspase-mediated cell death [5].

Accumulating evidence indicates that members of all three subclasses of the BCL-2 family of proteins are also located at the endoplasmic reticulum (ER) membrane (see list in [6]). The ER is an organelle with multiple functions, including lipid synthesis and signaling. The two major roles of the ER are calcium storage and protein folding. Membrane-spanning and secreted proteins are synthesized and folded in the ER, undergoing post-translational modifications and oligomerization. Several conditions (such as proteasome inhibition, mutant-protein expression, ER-calcium depletion and redox changes) interfere with oxidative protein folding at the ER lumen [7], resulting in the accumulation of unfolded or misfolded intermediates, a cellular condition referred to as 'ER stress'.

To alleviate ER stress, cells activate a complex signaling pathway known as the 'unfolded protein response' (UPR). The UPR transmits information about the protein folding status in the ER lumen to the cytoplasm and the nucleus to decrease the unfolded protein load. Activation of the UPR affects the expression of proteins involved in nearly every aspect of the secretory pathway, including protein entry into the ER, folding, ER-associated degradation, ER biogenesis and vesicular trafficking [7]. However, if all these mechanisms of survival are insufficient to decrease the unfolded protein load, cells enter into apoptosis. Therefore, improper handling of ER stress constitutes a threat to the life of the cell. The UPR/ER-stress pathway has been implicated in many diseases, including neurodegenerative conditions, cancer and diabetes [7]. Increasing evidence suggests the BCL-2 family of proteins has key roles at the ER membrane where these proteins modulate the cellular responses to stress in addition to cell death. In this review, we focus on the alternative roles for the BCL-2 family of proteins at the ER membrane.

Corresponding authors: Hetz, C. (chetz@med.uchile.cl); Glimcher, L. (lglimche@hsph.harvard.edu).

### The unfolded-protein response

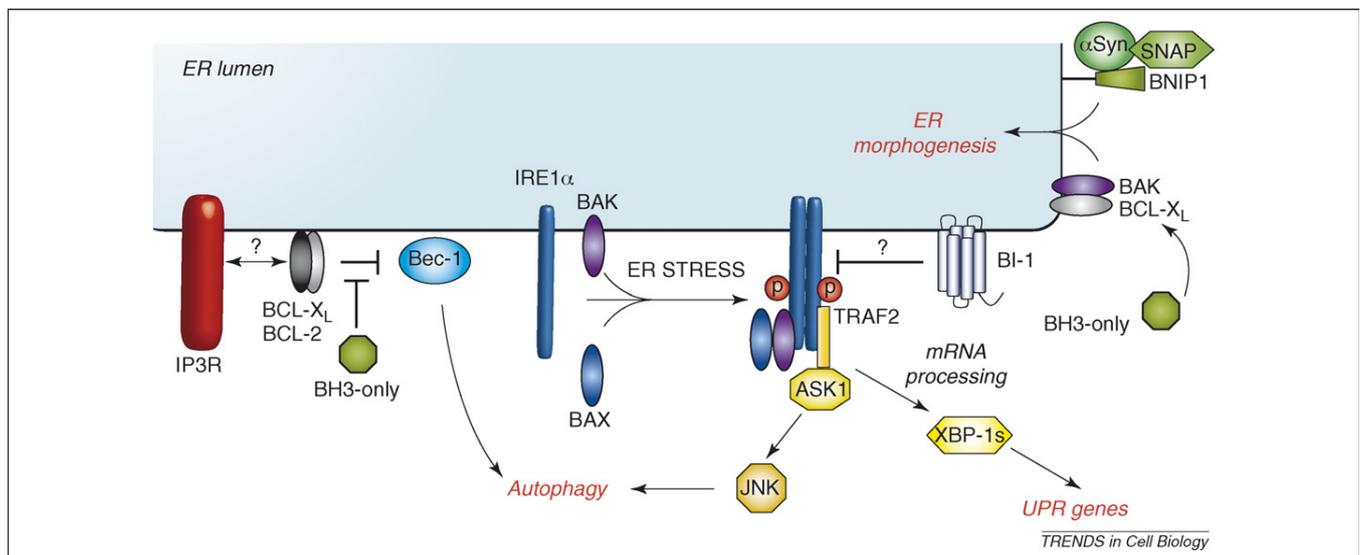
In higher eukaryotes, the UPR is mediated by at least three distinct UPR signaling pathways initiated by the sensors IRE1 $\alpha$  (inositol-requiring transmembrane kinase/ endonuclease), PERK (PKR-like ER kinase) and ATF6 (activating transcription factor 6) (reviewed in [8]). IRE1 $\alpha$  is a Ser/Thr protein kinase and endoribonuclease that, on activation, initiates the processing of the mRNA encoding XBP-1 (transcriptional factor X-Box-binding protein 1) [9–11]. A 26-nucleotide fragment of *xbp-1* mRNA is spliced out by activated IRE1 $\alpha$ , shifting the coding-reading frame of XBP-1, and resulting in the production of a stable and potent transcriptional activator called XBP-1s that controls the upregulation of a broad spectrum of UPR-related genes [12]. Indeed, XBP-1 production is essential for the proper function of specialized secretory organs, such as liver, pancreas, salivary gland and plasma B lymphocytes, in which a high rate of protein synthesis constitutes an endogenous source of stress [13–16]. IRE1 $\alpha$  activation also controls the activation of the c-Jun N-terminal kinase (JNK) [17,18], ERK [19] and NF- $\kappa$ B pathways [20], although their roles in the ER-stress response are not well understood.

### The BCL-2 protein family and the UPR

We provided evidence recently for a possible function of the BCL-2 protein family in the UPR (Figure 1). BAX and BAK modulate the amplitude of IRE1 $\alpha$  signaling by controlling its autophosphorylation and oligomerization [21]. BAX and BAK double-knockout (DKO) mice showed a decreased expression of IRE1 $\alpha$ -downstream signals, including JNK phosphorylation and XBP-1s expression under experimental ER-stress conditions [21]. At the biochemical level, BAX

and BAK form a protein complex with the cytosolic domain of IRE1 $\alpha$ , which requires their conserved BH1 and BH3 domains [21]. These findings suggested a new role for proapoptotic family members to act as accessory factors for the instigation of certain UPR signaling events. This implies that, during early steps of UPR responses, the proapoptotic proteins BAX and BAK might have pro-survival effects by promoting adaptation to ER stress. Thus, the adaptive effects of BAX and BAK against ER stress contrast completely with their known proapoptotic effect at the mitochondria, suggesting a compartmentalization of their function.

BAX inhibitor-1 (BI-1) is related functionally to the BCL-2 family of proteins and is located in the ER membrane primarily [22]. BI-1 has no obvious homology with BCL-2-related proteins, yet it interacts with different members of the family such as BCL-X<sub>L</sub> and BCL-2 [22,23]. Further studies revealed that BI-1 is well conserved in yeast, plants, viruses and many other organisms [24,25]. In mammalian cells, BI-1 is an antiapoptotic protein that affects ER-stress-dependent cell death partially, possibly by modulating calcium signaling [23] and oxidative-stress gene expression [26]. Interestingly, BI-1-deficient mice showed hyperactivation of the IRE1 $\alpha$  pathway *in vivo* in a model of hepatic and renal ischemia [27]. These results were also recapitulated recently in cellular models of ER stress [26] in which BI-1 overexpressing cells showed decreased UPR activation as evidenced by decreased XBP-1s. These data suggested that BI-1 has an inhibitory activity on the UPR, which contrasts with the opposite effect of BAX and BAK on this pathway. An interaction between BI-1 and IRE1 $\alpha$  remains to be determined. Based on these findings, we speculate that BI-1 has



**Figure 1.** Modulation of the UPR, autophagy and ER-membrane remodeling by the BCL-2 family of proteins. Regulation of the UPR: under stress conditions, IRE1 $\alpha$  dimerizes and autophosphorylates, leading to its activation. Phosphorylation of IRE1 $\alpha$  triggers its endoribonuclease activity, which mediates the processing of the mRNA encoding *XBP-1*. Alternatively, activated IRE1 $\alpha$  interacts with the adaptor protein TRAF2, leading to the activation of the JNK and NF- $\kappa$ B pathways. Activation of IRE1 $\alpha$  requires the binding of accessory proteins BAX and BAK, which might stabilize the active form of IRE1 $\alpha$ , augmenting its pro-survival signaling. The antiapoptotic protein BI-1 is located at the ER and has been suggested to be a negative regulator of the pathway. Regulation of ER remodeling: the interaction of BAK with BCL-X<sub>L</sub> at the ER membrane leads to drastic changes in ER structure. These effects might be modulated by some BH3-only proteins. The BH3-only protein BNIP1 forms a protein complex with Syntaxin-18 (Syn) and  $\alpha$ -SNARE, regulating ER-membrane remodeling and ER morphology. Autophagy: accumulation of misfolded proteins also triggers autophagy. Beclin-1 (Bec-1) is regulated negatively through an interaction with BCL-2 or BCL-X<sub>L</sub> at the ER membrane and this interaction is antagonized by BH3-only proteins. Activation of IRE1 $\alpha$  might increase the levels of autophagy through the activation of the JNK pathway. In addition, the IP3R controls autophagy that is dependent on BCL-2/BCL-XL expression.

an additional activity outside of the regulation of apoptosis at the ER, where it negatively controls IRE1 $\alpha$  activation.

We envision a model in which a complex protein platform operates at the ER membrane to control IRE1 $\alpha$  activity, an UPRosome, where multiple signaling responses are initiated (Figure 1). We predict that disruption of the interaction between BAX/BAK and IRE1 $\alpha$  might shut down the pro-survival effects of this pathway, sensitizing cells to apoptosis. It remains to be determined whether or not BH3-only proteins modulate the association of BAX and BAK to IRE1 $\alpha$ . Overall, this model proposes that the balance between anti- and pro-apoptotic members at the ER membrane might determine the ability of a cell to respond to ER stress by controlling the amplitude of UPR signaling.

### Irreversible ER stress: the killers

Chronic or irreversible ER stress results in apoptosis. It is not clear what is the initial signal or sensing mechanism that activates apoptosis in ER-damaged cells. However, many different factors have been identified as mediating caspase-dependent cell death downstream of ER stress [28]. Special attention has been focused on the identification of possible BH3-only proteins as mediators of ER-stress-induced apoptosis because the upstream signals activating BAX and BAK under ER stress are unclear.

The activity of BH3-only proteins has been studied mostly at the mitochondria, where they trigger cytochrome *c* release. BH3-only proteins can be separated functionally into two subtypes: (i) activators (i.e. BID, BIM and PUMA) that activate BAX and BAK directly to trigger cytochrome *c* release but are sequestered by antiapoptotic BCL-2 molecules; and (ii) sensitizers (i.e. BAD and NOXA) that only bind to and antagonize antiapoptotic BCL-2 members to release activator BH3-only proteins [29–31]. Alternatively, differential binding to antiapoptotic proteins might explain the separation between activator and sensitizer or derepressor BH3-only proteins [32].

Two BH3-only proteins, PUMA and NOXA, are induced strongly at the transcriptional level in cells undergoing prolonged ER stress. In a pioneering study, cDNA microarray analysis showed that PUMA is one of the only BH3 members that are upregulated by ER stress [33]. A global RNA-interference screen for genes that regulate ER-stress-mediated apoptosis corroborated the functional role of PUMA in this process and identified NOXA additionally as part of the pathway [34]. These data are further supported by the fact that *puma*- or *noxa*-deficient cells are partially resistant to apoptosis induced by ER injuries [35]. Another BH3-only member, BIK, is localized primarily to the ER [36]. BAK becomes oligomerized at the ER following BIK expression and BIK requires BAX/BAK to trigger calcium release and apoptosis (see next section) [37]. Another interesting example of an ER-linked BH3-only family member is BIM. Three different mechanisms link BIM to ER stress. Under normal conditions, BIM is found in the dynein motor complex of the microtubule cytoskeleton, whereas BIM translocates to the ER following ER-stress induction, where it might promote caspase activation through an unknown mechanism [38]. In addition, dephosphorylation of BIM by the phosphatase 2A under ER

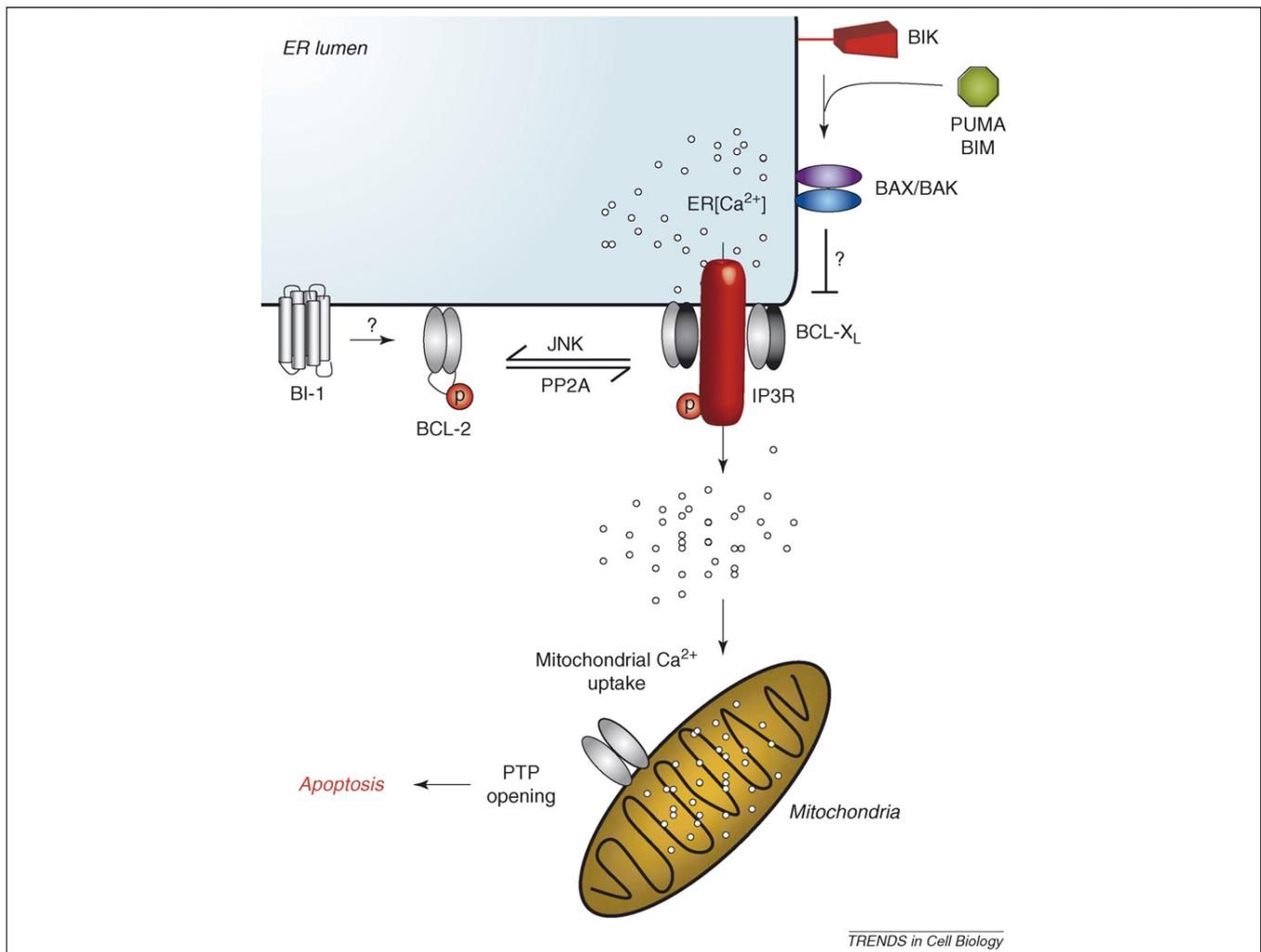
stress increases BIM levels by preventing its ubiquitination and subsequent proteasomal degradation in different cell types [39]. Further, expression of the proapoptotic UPR transcription factor C/EBP homologous protein (CHOP) triggers the upregulation of BIM mRNA. These results provided a direct connection between activation of the UPR and activation of the core proapoptotic program, a phenomenon that has remained mostly obscure. Moreover, BIM-deficient mice are resistant to ER-stress-induced apoptosis *in vivo*, similar to the phenotype described for *chop*-deficient mice [40,41]. In summary, irreversible ER damage triggers the upregulation of proapoptotic BH3-only proteins, which then might converge on the activation of BAX and BAK at the mitochondria, thus leading to cell death.

In murine cells, the processing of the ER-resident caspase-12 depends on the production of BAX and BAK at the ER [42]. Caspase-12 has been suggested to be linked to the UPR pathway through an interaction with tumor necrosis factor (TNF) receptor-associated factor-2 (TRAF2) and possibly with active IRE1 $\alpha$  [43], although a procaspase-12–TRAF2–IRE1 $\alpha$  complex has not been described. TRAF2 is known to be involved in the activation of initiator caspase-8 and caspase-10 through the death-receptor pathway. Although caspase-12 processing is a well established ER-stress marker, its contribution to apoptosis is debated actively [44,45].

### The BCL-2 protein family and the calcium rheostat

One of the main known functions described for the BCL-2 family of proteins at the ER is the control of calcium homeostasis (Figure 2). The balance between anti- and pro-apoptotic proteins at the ER determines the steady-state ER-calcium content and has a direct impact on the amount of calcium released after stimulation. For example, DKO cells for BAX and BAK show decreased ER-calcium content [42,46], similar to the phenotype of BCL-2 overexpressing cells [47]; overexpression of different BH3-only proteins triggers calcium release (reviewed in [6,48,49]). Thus, the BCL-2 family of proteins constitutes a rheostat for the fine tuning of calcium metabolism.

At the biochemical level, it has been documented by several groups that BCL-2 and BCL-X<sub>L</sub> form a protein complex with the inositol triphosphate receptor (IP3R) [50–52], modulating its on–off status possibly through phosphorylation (Figure 2). The IP3R, together with the ryanodine receptor, are the main channel that controls ER-calcium release in cells. Phosphorylation of BCL-2 by JNK occurs at the ER membrane. This modification regulates the antiapoptotic activity of BCL-2 negatively, correlating with a decreased binding to BH3-only proteins and increased ER-calcium content [47]. Oakes *et al.* also suggested that the calcium defects observed in BAX and BAK DKO cells might be attributed to their effect on the interaction of BCL-2 and the IP3R [50]. The native protein complexes containing BCL-2 at the ER membrane were purified recently [53]. BCL-2 is regulated directly by the serine/threonine phosphatase, PP2A [53], which dephosphorylates the sites targeted by JNK, suggesting that the phosphorylation status of BCL-2 is key to its regulation at the ER. Finally, as described earlier, different BH3-only proteins are located at the ER or translocate to its membrane under stress conditions and



**Figure 2.** Regulation of ER-calcium homeostasis by the BCL-2 family of proteins. Different anti- and pro-apoptotic members of the BCL-2 family of proteins are located at the ER membrane, where they regulate ER-calcium content. BCL-2 and BCL-X<sub>L</sub> interact with the IP3R calcium channel, modulating its activity. BCL-2 increases ER-calcium leak through the IP3 receptor (IP3R) owing to an increase of IP3R phosphorylation. By contrast, BAX and BAK have the opposite effect on ER-calcium content, a function that might be further modulated by BH3-only proteins (such as PUMA and BIK), possibly by affecting the interaction of BCL-2/BCL-X<sub>L</sub> with the IP3R indirectly. In addition, the activity of BCL-2 at the ER membrane is regulated by phosphorylation. JNK phosphorylates BCL-2, decreasing its antiapoptotic activity and increasing ER-calcium content, whereas the phosphatase PP2A decreases this phosphorylation through a direct interaction. BI-1 is also located at the ER membrane, where it regulates calcium homeostasis by an unknown mechanism. However, calcium release from the ER influences mitochondrial-mediated apoptosis through calcium uptake followed by the opening of the mitochondrial permeability-transition pore (PTP).

impact calcium homeostasis (reviewed in [6]). Thus, one can speculate that, depending on the cellular context and the stimuli, different protein complexes among BCL-2 family members might exist at the ER membrane to control calcium signaling. Hence, the balance between pro- and antiapoptotic proteins at the ER determines the ER-calcium content and rate of calcium release. Calcium release by the ER can activate apoptosis under certain conditions. For example, calcium uptake by the mitochondria induces mitochondrial membrane permeabilization owing to the opening of the permeability-transition pore (PTP). This crosstalk between the ER and mitochondria affects calcium-dependent cell death (i.e. ceramides, arachidonic acid or oxidative stress) specifically but not ER-stress-mediated apoptosis [46] (Figure 2).

#### ER signaling, autophagy and the BCL-2 protein family

Autophagy refers to the global process by which intracellular components are recycled through lysosome

degradation (reviewed in [54]). Autophagy acts as a crucial survival response under starvation conditions in which the degradation of intracellular proteins and organelles provides a source of amino acids during poor nutritional conditions. Intracellular components can be delivered to lysosomes for degradation by three different mechanisms known as macroautophagy, microautophagy and chaperone-mediated autophagy [54]. The best studied form of autophagy is macroautophagy, hereafter referred to as autophagy. The hallmark of autophagy is the formation of double-membrane-bounded autophagosomes. Autophagosomes fuse with lysosomes to form autophagolysosomes, in which intracellular components are degraded. Autophagy is a highly regulated process with complex steps that are controlled by a family of autophagic-related genes (termed *atg* genes) [54,55]. The generation of *atg*-deficient mice revealed the function of autophagy in diverse processes, including development, cell differentiation, tissue remodeling, immunity,

host-to-pathogen response and cell death or survival under stress conditions [54].

Beclin-1 (also known as Atg6) was the first identified mammalian autophagy gene product [56]. Beclin-1 was isolated originally as a BCL-2-interacting protein [57–59]. In fact, the BCL-2 was shown to negatively regulate autophagy through inhibiting Beclin-1 [60]. Surprisingly, this regulation was attributed specifically to the expression of BCL-2 at the ER membrane, suggesting that signaling events originating from the ER are crucial for autophagy. The formation of a BCL-2–Beclin-1 complex is also regulated by BH3-only proteins (i.e. BAD), revealing extensive crosstalk between apoptosis and autophagy [61,62]. More importantly, a functional BH3-like domain was identified in Beclin-1 and its mutation disrupted the interaction of Beclin-1 with BCL-X<sub>L</sub> [61]. Experiments performed in *Caenorhabditis elegans* deficient in EGL-1, a BH3-containing protein, corroborated this model *in vivo*. Similarly, the pharmacological BH3 mimetic ABT-737 inhibited the interaction between Beclin-1 and Bcl-2/Bcl-X<sub>L</sub> competitively, stimulating autophagy [61,62].

Along the same lines, a connection between ER-calcium homeostasis and autophagy was proposed to occur through the IP3R, a BCL-2 interactor as described earlier [63–65] (reviewed in [66]). IP3R-dependent autophagy was attributed to the activation of Beclin-1 and other autophagic-related genes, however, unexpectedly, this regulation was independent of calcium release. Finally, many laboratories have shown that ER stress triggers autophagy and this effect is also regulated by UPR stress sensors [63,67–72]. Autophagy might serve as a mechanism to eliminate damaged ER under stress conditions or to control the rate of ER expansion [70]. Surprisingly, the activation of autophagy by ER stress requires IRE1 $\alpha$  and is not inhibited by BCL-2 overexpression, a strategy that blocks autophagy mediated by IP3R inhibition. Along the same lines, the IP3R regulates autophagy independently of IRE1 $\alpha$ , suggesting that there are two independent pathways controlling autophagy from the ER [63]. It remains to be established whether or not a direct connection among the UPR, autophagy and the BCL-2 protein family exists.

### The BCL-2 protein family and ER morphogenesis

The expression of some proapoptotic members of the BCL-2 family of proteins alters the structure of the ER. BAK regulates ER swelling and the remodeling of the reticular structure [73]. Co-expression of BAK and BCL-X<sub>L</sub> or BAK mutants in the BH3 domain provoked extensive swelling and vacuolization of ER cisternae. Interestingly, the co-expression of upstream BH3-only activators (i.e. BIM or truncated BID) with BCL-X<sub>L</sub> recapitulated ER swelling and vacuolization under conditions in which the ryanodine receptor-calcium channel is inhibited [73]. Surprisingly, the effects of BH3-only proteins on ER swelling were proposed to be dependent on BAK expression specifically but not on BAX, the closest BAK homologue. The physiological role of ER swelling in this context is not known.

The BH3-only member BNip1 is a component of a protein complex comprising syntaxin 18, an ER-located soluble N-ethylmaleimide-sensitive factor (NSF)-attachment protein receptor (SNARE) [74]. At the functional

level, BNip1 participates in the formation and modeling of the ER network, being involved in ER membrane fusion but not membrane trafficking. In addition, the BH3 domain is important for the binding of BNip1 to the SNAREs [74]. These results unmasked a possible crosstalk between apparently independent cellular events, apoptosis and ER-membrane fusion (Figure 1). Alternatively, BAX and BAX might also regulate ER biogenesis through XBP-1 [21]. Similarly, expression of BAX and BAK are essential to mitochondria morphogenesis [75], suggesting that these proteins might have broad regulatory effects on organelle morphogenesis.

### Concluding remarks

In this review, we have summarized evidence supporting an evolutionary process whereby key regulators of cell death also contribute to vital cellular functions. Accordingly, BCL-2-related proteins not only operate as upstream regulators of caspases but they also regulate specific cellular processes related to ER physiology actively. In support of this idea, there is growing evidence of nonapoptotic functions for the BCL-2 family, including cell-cycle regulation [76,77], participation in DNA-damage responses (i.e. BID) [78,79] and glucose or energy metabolism (i.e. BAD) [80]. In doing so, the BCL-2 protein family might perform as specialized stress sentinels that participate actively in crucial processes for the cell ('the day job'), enabling constant homeostatic quality control. Then the BCL-2 protein family could respond to irreversible cellular damage, activating apoptosis ('the killer properties').

Misfolding and accumulation of abnormal protein aggregates in the brain owing to mutations in certain genes are responsible for a variety of neurological disorders. In many of these diseases, alteration of ER homeostasis contributes to neuronal dysfunction. These diseases include Parkinson's disease [81,82], Alzheimer's disease [83], prion diseases [84–86], amyotrophic lateral sclerosis (ALS) [87], Huntington's disease [88,89] and many others (see list of diseases in [7]). Consequently, the first steps in the death pathways downstream of ER stress might represent important therapeutic targets. In addition, BH3-only proteins, such as BIM, have been implicated in Alzheimer's disease [90] and ALS [91] *in vivo*. Thus, pharmacological manipulation of the BCL-2 protein family activity might be beneficial in the treatment of these fatal diseases. Several small molecules and synthetic peptides are available currently with proven therapeutic applications in disease mouse models, including BCL-2 inhibitors [92], BAX-channel inhibitors [93], BAX/BAK-activator peptides [94,95] and many others (see reviews in [5,96]). These drugs might be used as pharmacological tools to manipulate the activity of stress-signaling pathways regulated by the BCL-2 protein family (i.e. autophagy, calcium, metabolism or the UPR) in pathological conditions.

### Acknowledgements

This work was supported by the V Harold and Leila Y Mathers Charitable Foundation and NIH grant AI32412 and AI56296 (LHG); and FONDECYT no. 1070444, FONDAP grant no.15010006 and High Q Foundation (CH). We thank Anthony Letai for suggesting the title.

## References

- 1 Danial, N.N. and Korsmeyer, S.J. (2004) Cell death: critical control points. *Cell* 116, 205–219
- 2 Zhang, H.M. *et al.* (2003) BNIPs: a group of pro-apoptotic proteins in the Bcl-2 family. *Apoptosis* 8, 229–236
- 3 Labi, V. *et al.* (2006) BH3-only proteins in cell death initiation, malignant disease and anticancer therapy. *Cell Death Differ.* 13, 1325–1338
- 4 Wei, M.C. *et al.* (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* 292, 727–730
- 5 Reed, J.C. (2006) Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. *Cell Death Differ.* 13, 1378–1386
- 6 Oakes, S.A. *et al.* (2006) The control of endoplasmic reticulum-initiated apoptosis by the BCL-2 family of proteins. *Curr. Mol. Med.* 6, 99–109
- 7 Schroder, M. and Kaufman, R.J. (2005) The mammalian unfolded protein response. *Annu. Rev. Biochem.* 74, 739–789
- 8 Ron, D. and Walter, P. (2007) Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* 8, 519–529
- 9 Lee, K. *et al.* (2002) IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev.* 16, 452–466
- 10 Calton, M. *et al.* (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415, 92–96
- 11 Yoshida, H. *et al.* (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107, 881–891
- 12 Lee, A.H. *et al.* (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol. Cell. Biol.* 23, 7448–7459
- 13 Reimold, A.M. *et al.* (2000) An essential role in liver development for transcription factor XBP-1. *Genes Dev.* 14, 152–157
- 14 Reimold, A.M. *et al.* (2001) Plasma cell differentiation requires the transcription factor XBP-1. *Nature* 412, 300–307
- 15 Iwakoshi, N.N. *et al.* (2003) Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. *Nat. Immunol.* 4, 321–329
- 16 Lee, A.H. *et al.* (2005) XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. *EMBO J.* 24, 4368–4380
- 17 Urano, F. *et al.* (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287, 664–666
- 18 Nishitoh, H. *et al.* (2002) ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev.* 16, 1345–1355
- 19 Nguyen, D.T. *et al.* (2004) Nck-dependent activation of extracellular signal-regulated kinase-1 and regulation of cell survival during endoplasmic reticulum stress. *Mol. Biol. Cell* 15, 4248–4260
- 20 Hu, P. *et al.* (2006) Autocrine tumor necrosis factor  $\alpha$  links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1 $\alpha$ -mediated NF- $\kappa$ B activation and down-regulation of TRAF2 expression. *Mol. Cell. Biol.* 26, 3071–3084
- 21 Hetz, C. *et al.* (2006) Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1 $\alpha$ . *Science* 312, 572–576
- 22 Xu, Q. and Reed, J.C. (1998) Bax inhibitor-1, a mammalian apoptosis suppressor identified by functional screening in yeast. *Mol. Cell* 1, 337–346
- 23 Chae, H.J. *et al.* (2004) BI-1 regulates an apoptosis pathway linked to endoplasmic reticulum stress. *Mol. Cell* 15, 355–366
- 24 Chae, H.J. *et al.* (2003) Evolutionarily conserved cytoprotection provided by Bax inhibitor-1 homologs from animals, plants, and yeast. *Gene* 323, 101–113
- 25 Huckelhoven, R. (2004) BAX Inhibitor-1, an ancient cell death suppressor in animals and plants with prokaryotic relatives. *Apoptosis* 9, 299–307
- 26 Lee, G.H. *et al.* (2007) Bax inhibitor-1 regulates endoplasmic reticulum stress-associated reactive oxygen species and heme oxygenase-1 expression. *J. Biol. Chem.* 282, 21618–21628
- 27 Bailly-Maitre, B. *et al.* (2006) Cytoprotective gene *bi-1* is required for intrinsic protection from endoplasmic reticulum stress and ischemia-reperfusion injury. *Proc. Natl. Acad. Sci. U. S. A.* 103, 2809–2814
- 28 Szegezdi, E. *et al.* (2006) ER stress contributes to ischemia-induced cardiomyocyte apoptosis. *Biochem. Biophys. Res. Commun.* 349, 1406–1411
- 29 Kim, H. *et al.* (2006) Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. *Nat. Cell Biol.* 8, 1348–1358
- 30 Letai, A. *et al.* (2002) Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell* 2, 183–192
- 31 Kuwana, T. *et al.* (2005) BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. *Mol. Cell* 17, 525–535
- 32 Willis, S.N. *et al.* (2007) Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* 315, 856–859
- 33 Reimertz, C. *et al.* (2003) Gene expression during ER stress-induced apoptosis in neurons: induction of the BH3-only protein Bbc3/PUMA and activation of the mitochondrial apoptosis pathway. *J. Cell Biol.* 162, 587–597
- 34 Futami, T. *et al.* (2005) Identification of a network involved in thapsigargin-induced apoptosis using a library of small interfering RNA expression vectors. *J. Biol. Chem.* 280, 826–831
- 35 Li, J. *et al.* (2006) Endoplasmic reticulum stress-induced apoptosis: multiple pathways and activation of p53-up-regulated modulator of apoptosis (PUMA) and NOXA by p53. *J. Biol. Chem.* 281, 7260–7270
- 36 Germain, M. *et al.* (2002) BH-3-only BIK functions at the endoplasmic reticulum to stimulate cytochrome c release from mitochondria. *J. Biol. Chem.* 277, 18053–18060
- 37 Mathai, J.P. *et al.* (2005) BH3-only BIK regulates BAX, BAK-mediated release of Ca<sup>2+</sup> from endoplasmic reticulum stores and mitochondrial apoptosis during stress-induced cell death. *J. Biol. Chem.* 280, 23829–23836
- 38 Morishima, N. *et al.* (2004) Translocation of Bim to the endoplasmic reticulum (ER) mediates ER stress signaling for activation of caspase-12 during ER stress-induced apoptosis. *J. Biol. Chem.* 279, 50375–50381
- 39 Puthalakath, H. *et al.* (2007) ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* 129, 1337–1349
- 40 Marciniak, S.J. *et al.* (2004) CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.* 18, 3066–3077
- 41 Zinszner, H. *et al.* (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev.* 12, 982–995
- 42 Zong, W.X. *et al.* (2003) Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis. *J. Cell Biol.* 162, 59–69
- 43 Yoneda, T. *et al.* (2001) Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J. Biol. Chem.* 276, 13935–13940
- 44 Scott, A.M. and Saleh, M. (2006) The inflammatory caspases: guardians against infections and sepsis. *Cell Death Differ.* 14, 23–31
- 45 Saleh, M. *et al.* (2006) Enhanced bacterial clearance and sepsis resistance in caspase-12-deficient mice. *Nature* 440, 1064–1068
- 46 Scorrano, L. *et al.* (2003) BAX and BAK regulation of endoplasmic reticulum Ca<sup>2+</sup>: a control point for apoptosis. *Science* 300, 135–139
- 47 Bassik, M.C. *et al.* (2004) Phosphorylation of BCL-2 regulates ER Ca<sup>2+</sup> homeostasis and apoptosis. *EMBO J.* 23, 1207–1216
- 48 Breckenridge, D.G. *et al.* (2003) Regulation of apoptosis by endoplasmic reticulum pathways. *Oncogene* 22, 8608–8618
- 49 Pinton, P. and Rizzuto, R. (2006) Bcl-2 and Ca<sup>2+</sup> homeostasis in the endoplasmic reticulum. *Cell Death Differ.* 13, 1409–1418
- 50 Oakes, S.A. *et al.* (2004) Proapoptotic BAX and BAK regulate the type 1 inositol triphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U. S. A.* 102, 105–110
- 51 Chen, R. *et al.* (2004) Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. *J. Cell Biol.* 166, 193–203
- 52 White, C. *et al.* (2005) The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. *Nat. Cell Biol.* 7, 1021–1028
- 53 Lin, S.S. *et al.* (2006) PP2A regulates BCL-2 phosphorylation and proteasome-mediated degradation at the endoplasmic reticulum. *J. Biol. Chem.* 281, 23003–23012

- 54 Levine, B. (2005) Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell* 120, 159–162
- 55 Cuervo, A.M. (2004) Autophagy: in sickness and in health. *Trends Cell Biol.* 14, 70–77
- 56 Aita, V.M. *et al.* (1999) Cloning and genomic organization of *beclin 1*, a candidate tumor suppressor gene on chromosome 17q21. *Genomics* 59, 59–65
- 57 Liang, X.H. *et al.* (1999) Induction of autophagy and inhibition of tumorigenesis by *beclin 1*. *Nature* 402, 672–676
- 58 Qu, X. *et al.* (2003) Promotion of tumorigenesis by heterozygous disruption of the *beclin 1* autophagy gene. *J. Clin. Invest.* 112, 1809–1820
- 59 Yue, Z. *et al.* (2003) *Beclin 1*, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc. Natl. Acad. Sci. U. S. A.* 100, 15077–15082
- 60 Pattingre, S. *et al.* (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 122, 927–939
- 61 Maiuri, M.C. *et al.* (2007) Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. *EMBO J.* 26, 2527–2539
- 62 Maiuri, M.C. *et al.* (2007) BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X(L). *Autophagy* 3, 374–376
- 63 Criollo, A. *et al.* (2007) The inositol trisphosphate receptor in the control of autophagy. *Autophagy* 3, 350–353
- 64 Criollo, A. *et al.* (2007) Regulation of autophagy by the inositol trisphosphate receptor. *Cell Death Differ.* 14, 1029–1039
- 65 Hoyer-Hansen, M. *et al.* (2007) Control of macroautophagy by calcium, calmodulin-dependent kinase kinase- $\beta$ , and Bcl-2. *Mol. Cell* 25, 193–205
- 66 Hoyer-Hansen, M. and Jaattela, M. (2007) Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ.* 14, 1576–1582
- 67 Kouroku, Y. *et al.* (2006) ER stress (PERK/eIF2 $\alpha$  phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. *Cell Death Differ.* 14, 230–239
- 68 Ogata, M. *et al.* (2006) Autophagy is activated for cell survival after ER stress. *Mol. Cell Biol.* 26, 9220–9231
- 69 Yorimitsu, T. *et al.* (2006) Endoplasmic reticulum stress triggers autophagy. *J. Biol. Chem.* 281, 30299–30304
- 70 Bernales, S. *et al.* (2006) Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biol.* 4, e423
- 71 Ding, W.X. *et al.* (2007) Linking of autophagy to ubiquitin-proteasome system is important for the regulation of endoplasmic reticulum stress and cell viability. *Am. J. Pathol.* 171, 513–524
- 72 Ding, W.X. *et al.* (2007) Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. *J. Biol. Chem.* 282, 4702–4710
- 73 Klee, M. and Pimentel-Muinos, F.X. (2005) Bcl-X(L) specifically activates Bak to induce swelling and restructuring of the endoplasmic reticulum. *J. Cell Biol.* 168, 723–734
- 74 Nakajima, K. *et al.* (2004) Involvement of BNIP1 in apoptosis and endoplasmic reticulum membrane fusion. *EMBO J.* 23, 3216–3226
- 75 Karbowski, M. *et al.* (2006) Role of Bax and Bak in mitochondrial morphogenesis. *Nature* 443, 658–662
- 76 Zinkel, S. *et al.* (2006) BCL2 family in DNA damage and cell cycle control. *Cell Death Differ.* 13, 1351–1359
- 77 Schuler, M. and Green, D.R. (2005) Transcription, apoptosis and p53: catch-22. *Trends Genet.* 21, 182–187
- 78 Zinkel, S.S. *et al.* (2005) A role for proapoptotic BID in the DNA-damage response. *Cell* 122, 579–591
- 79 Kamer, I. *et al.* (2005) Proapoptotic BID is an ATM effector in the DNA-damage response. *Cell* 122, 593–603
- 80 Danial, N.N. *et al.* (2003) BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. *Nature* 424, 952–956
- 81 Ryu, E.J. *et al.* (2002) Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. *J. Neurosci.* 22, 10690–10698
- 82 Holtz, W.A. and O'Malley, K.L. (2003) Parkinsonian mimetics induce aspects of unfolded protein response in death of dopaminergic neurons. *J. Biol. Chem.* 278, 19367–19377
- 83 Ghribi, O. (2006) The role of the endoplasmic reticulum in the accumulation of beta-amyloid peptide in Alzheimer's disease. *Curr. Mol. Med.* 6, 119–133
- 84 Hetz, C. *et al.* (2003) Caspase-12 and endoplasmic reticulum stress mediate neurotoxicity of pathological prion protein. *EMBO J.* 22, 5435–5445
- 85 Hetz, C. *et al.* (2005) The disulfide isomerase Grp58 is a protective factor against prion neurotoxicity. *J. Neurosci.* 25, 2793–2802
- 86 Hetz, C.A. and Soto, C. (2006) Stressing out the ER: a role of the unfolded protein response in prion-related disorders. *Curr. Mol. Med.* 6, 37–43
- 87 Turner, B.J. and Atkin, J.D. (2006) ER stress and UPR in familial amyotrophic lateral sclerosis. *Curr. Mol. Med.* 6, 79–86
- 88 Sekine, Y. *et al.* (2006) The ASK1-MAP kinase signaling in ER stress and neurodegenerative diseases. *Curr. Mol. Med.* 6, 87–97
- 89 Momoi, T. (2006) Conformational diseases and ER stress-mediated cell death: apoptotic cell death and autophagic cell death. *Curr. Mol. Med.* 6, 111–118
- 90 Biswas, S.C. *et al.* (2007) Bim is elevated in Alzheimer's disease neurons and is required for beta-amyloid-induced neuronal apoptosis. *J. Neurosci.* 27, 893–900
- 91 Hetz, C. *et al.* (2007) The proapoptotic BCL-2 family member BIM mediates motoneuron loss in a model of amyotrophic lateral sclerosis. *Cell Death Differ.* 14, 1386–1389
- 92 Oltersdorf, T. *et al.* (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 435, 677–681
- 93 Hetz, C. *et al.* (2005) Bax channel inhibitors prevent mitochondrion-mediated apoptosis and protect neurons in a model of global brain ischemia. *J. Biol. Chem.* 280, 42960–42970
- 94 Walensky, L.D. *et al.* (2006) A stapled BID BH3 helix directly binds and activates BAX. *Mol. Cell* 24, 199–210
- 95 Walensky, L.D. *et al.* (2004) Activation of apoptosis *in vivo* by a hydrocarbon-stapled BH3 helix. *Science* 305, 1466–1470
- 96 Letai, A. (2005) Pharmacological manipulation of Bcl-2 family members to control cell death. *J. Clin. Invest.* 115, 2648–2655