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Forum Review

ER Stress Signaling and the BCL-2 Family of Proteins: From Adaptation to Irreversible Cellular Damage

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ABSTRACT

Programmed cell death is essential for the development and maintenance of cellular homeostasis, and its deregulation results in a variety of pathologic conditions. The BCL-2 family of proteins is a group of evolutionarily conserved regulators of cell death that operate at the mitochondrial membrane to control caspase activation. This family is comprised both of antiapoptotic and proapoptotic members, in which a subset of proapoptotic members, called BH3-only proteins, acts as upstream activators of the core proapoptotic pathway. In addition to their known role at the mitochondria, different BCL-2–related proteins are located to the endoplasmic reticulum (ER) membrane, where new functions have been recently proposed. In this review, evidence is presented indicating that members of the BCL-2 protein family are contained in multiprotein complexes at the ER, regulating diverse cellular processes including autophagy, calcium homeostasis, the unfolded-protein response, ER membrane remodeling, and calcium-dependent cell death. Thus, BCL-2–related proteins are not only the "death gateway" keepers, but they also have alternative functions in essential cellular processes. *Antioxid. Redox Signal.* 9, 2345–2355.

INTRODUCTION

Eukaryotic cells have evolved sophisticated molecular responses to overcome perturbations on cellular homeostasis, in which the dynamic integration between the function of different organelles is a key component of *autopoiesis* (19, 99). At each organelle, different molecular sentinels permanently sense stressful cellular conditions and initiate complex signaling responses, either to adapt to the new conditions or to activate specific cell death–signaling pathways if a critical threshold of damage has been reached (19). Cell demise through apoptosis ultimately depends on the activation of cysteine proteases of the caspase family and other proteases (65) that execute cell death. Apoptosis is a genetically controlled form of cell suicide that is essential for successful development and tissue homeostasis of all multicellular organisms (72). Several intracellular apoptosis-signaling pathways have been identified, including the death-receptor pathway and the mitochondria-dependent pathway [reviews in (17, 72)]. The BCL-2 family proteins are located at organelle membranes, representing upstream regulators of caspases (17). This family consists on a large group of proteins comprising pro- and antiapoptotic members and is defined by the presence of up to four small conserved domains within their primary structure (Figs. 1 and 2). Antiapoptotic BCL-2 family members display sequence homology in four α-helical domains called BCL-2 homology (BH)1 to BH4 (17). Proapoptotic BCL-2 members can be further subdivided into more fully conserved, "multidomain" members containing homology in the BH1, BH2, and BH3 (*i.e.*, BAX and BAK) or the "BH3-only" members (*i.e.*, BID, BIK, BIM, PUMA, and NOXA), which contain a single α-helical domain critical for activation of apoptosis (see Figs. 1 and 2) [see substantial reviews about different family members in (2, 6, 7, 17, 68, 72, 76, 79, 95)]. This pathway has gained complexity since the identification of a new subgroup of proapoptotic proteins containing poor conservation in the BH3 domain, called BNip proteins (review in 111). Increasing evidence suggests that BNips may play an active role in cell death and other cellular

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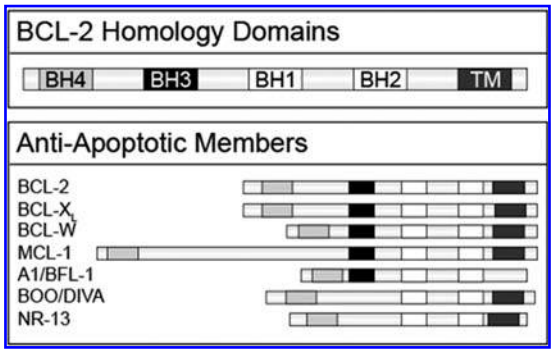

FIG. 1. The anti-apoptotic BCL-2 family members. The BCL-2 family of proteins is defined by the presence of up to four homology domains with its former member BCL-2. This group of proteins is functionally subdivided into two main components, pro- and antiapoptotic proteins. Major antiapoptotic proteins are presented. *Black box*, conserved BH3 domain. *Gray box*, predicted transmembrane regions (TMs).

processes; however, the precise mechanisms by which the BNips induce apoptosis remain elusive.

Each member of the BCL-2 family has distinct patterns of developmental expression, subcellular localization, and differential responsiveness to specific death stimuli (review in 48). In general, it is possible to envision a simplified model of how the BCL-2 protein family regulates apoptosis: intrinsic death stimuli are highly dependent on particular BH3-only proteins to trigger apoptosis, in which they can act as sentinels of irreversible cellular damage or be activated by cell-fate or developmental programs (17). BH3-only proteins are activated either by transcriptional upregulation or through post-translational modifications. Once activated, the BH3-only proteins converge on the activation of multidomain proapoptotic proteins BAX and/or BAK, which function in concert as an essential gateway to the intrinsic cell death pathways operating at the mitochondria (102). Activation of BAX and BAK is mediated by their intramembranous oligomerization and resultant permeabilization of the mitochondrial outer membrane (17). Released mitochondrial proteins, such as cytochrome *c*, trigger the activation of caspases through the formation of the apoptosome complex (79).

Recent evidence indicates that members of all three subclasses of the BCL-2 family of proteins are also located to the endoplasmic reticulum (ER) (68). The ER is a subcellular compartment with multiple functions. For example, the ER is the location where the biosynthesis of steroids, cholesterol, and other lipids occurs, playing a crucial role in organelle biogenesis and signaling through the generation of lipid second messengers. The ER is well known as a major calcium store in the cells, and thus constitutes a signaling organelle that can modulate many calcium-dependent processes such as proliferation, cell death, and differentiation (review in 8). The ER is also the site where membrane-spanning and secreted proteins are synthesized; facilitating many protein post-translational modifications, in addition to their folding and proper oligomerization. A number of conditions can interfere with oxidative protein folding at the ER lumen (86). These perturbations foment the accumulation of unfolded or misfolded proteins, a cellular condition referred to as "ER stress." Therefore, improper handling of ER stress constitutes a threat to the life of the cell.

To alleviate the stress in the ER, cells activate a complex intracellular signaling pathway known as the "unfolded protein response" (UPR). To decrease the unfolded protein load, the UPR transmits information about the protein-folding status in the ER lumen to the cytoplasm and the nucleus. In doing so, 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, glycosylation, ER-associated degradation, ER biogenesis, lipid metabolism, and vesicular trafficking (reviewed in 18, 86). Increasing attention has been given to the function of the ER based on substantial reports suggesting the involvement of the UPR/ER stress pathway in many diseases, including neurodegenerative conditions (77), cancer (44), and diabetes (59). In this review, I focus my attention on the discussion of new findings suggesting alternative roles for the BCL-2 family of proteins at the ER membrane.

THE BCL-2 PROTEIN FAMILY AND THE ER GATEWAY OF DEATH

Several members of the BCL-2 family localize at the ER membrane, and a growing appreciation exists that they may actively control apoptosis from this organelle. BAX and BAK localize to the ER, and double knockout BAX/BAK (DKO) cells are highly resistant to death stimuli dependent on ER-calcium release (88) and ER stress injuries (102, 116). In addition, ER stress triggers the oligomerization of BAX and BAK at the ER (116). The antiapoptotic members BCL-2 or BCL-X_L are also

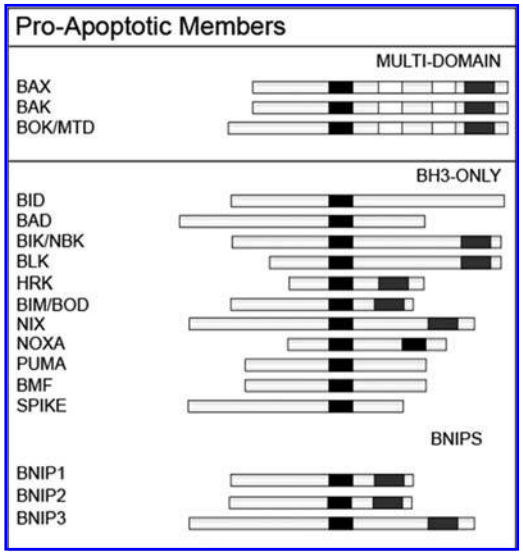

FIG. 2. The proapoptotic BCL-2 family members. Proapoptotic members can be further subdivided into more fully conserved "multidomain" members (such as BAX and BAK), possessing BCL-2 homology domains 1, 2, and 3, or "BH3-only" members (such as BIM, PUMA, and BNIPs), which display only about nine amino acids of sequence homology within this single death-promoting domain, BCL-2 homology domains 1 to 4 (BH1-4). *Black box*, conserved BH3 domain. *Gray box*, predicted transmembrane regions (TMs).

localized at the ER, and their overexpression reduces ER stress–mediated apoptosis (4, 64, 83).

One of the main known functions described for the BCL-2 family of proteins at the ER is the control of calcium homeostasis. It has been proposed that the balance between anti- and proapoptotic proteins at the ER determine the calcium content of this organelle, having a direct impact on the amount of calcium released after stimulation (Fig. 3). For example, DKO cells for BAX and BAK show decreased ER calcium content (88, 116), similar to the phenotype of BCL-2–overexpressing cells (4). In addition, overexpression of different proapoptotic BCL-2 family members triggers calcium release (review in 7, 68, 74). Conversely, BCL-2–deficient cells show an increased ER calcium content and increased calcium release from the ER after stimulation (4). 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_L form a protein complex with the inositol triphosphate receptor (IP3R) (12, 69, 104), modulating its activity. Oakes *et al.* (98) also suggested that the calcium-leak defects observed in BAX and BAK DKO cells may be explained by increased "free levels" of BCL-2 at the ER, increasing its interaction with IP3R and its phosphorylation state (69).

Phosphorylation of BCL-2 by the c-Jun N-terminal kinase (JNK) has been shown to occur at the ER membrane during the cell cycle at the G_2/M stage. This phosphorylation event negatively regulates its antiapoptotic activity, in addition to its binding to BH3-only proteins and the ER calcium content (4). Lin and co-workers recently purified the native protein complexes containing BCL-2 at the ER (58). Surprisingly, they found that BCL-2 interacts with the main two subunits of the serine/threonine phosphatase, PP2A. PP2A was shown to regulate BCL-2 antiapoptotic activity at the ER through dephosphorylation (58). Finally, different BH3-only proteins are located at the ER or translocate to its membrane under ER stress conditions, having an impact on calcium homeostasis (review in 68). Thus, it is possible to speculate that, depending on the cellular context and the stimuli, different multiprotein complexes between particular BCL-2 family members may exist at the ER membrane to control calcium signaling (see Fig. 3).

Despite clear evidence linking the function of the BCL-2 protein family and calcium homeostasis, the mechanism by which these proteins modulate the activation of apoptosis from the ER is not well understood, and some of the available data have also been misunderstood by the community. Scorrano *et al.* demonstrated with a series of elegant experiments that the regulation of calcium signaling by BAX and BAK specifically affects calcium-dependent cell death (*i.e.,* ceramide, arachidonic acid, or H_2O_2 toxicity) and not ER stress-mediated apoptosis (88). In this case, differential calcium release from the ER has a direct impact in the amount of calcium uptake by the mitochondria, triggering membrane permeabilization. This is an important distinction because the expression of BAX and BAK at the mitochondria is essential for the activation of apoptosis under irreversible ER stress, suggesting the existence of a cross-talk between ER- and mitochondrial-located BCL-2–related proteins. Consequently, it may be possible that the role of the BCL-2 protein family on ER calcium homeostasis reflects a different function for these proteins rather than a mechanism for the regulation of apoptosis after ER damage. It is not well understood how the cross-talk between the ER and the mitochondria occurs to induce ER stress–mediated apoptosis. The movement of a "soluble factor" from the ER to the mitochondria has been suggested (96). However, it is most likely that upregulation of some BH3-only proteins at transcriptional levels may result in the direct engagement of BAX/BAK activation at the mitochondria, triggering cell death (see later).

ER STRESS–MEDIATED APOPTOSIS: THE BH3-ONLY CONNECTION

The activity of BH3-only proteins has been studied mainly at the mitochondria, where they trigger cell death. BH3-only proteins can be functionally separated into two subtypes: (a) *activators* (*i.e.*, BID, BIM, and PUMA) that can bind to antiapoptotics, but also directly activate BAX and BAK to trigger cytochrome *c* release; and (b) *sensitizers* (*i.e.*, BAD and NOXA) that only bind and antagonize antiapoptotic BCL-2 members to sequester them away from interfering with the activation of BAX and BAK (39, 47, 53).

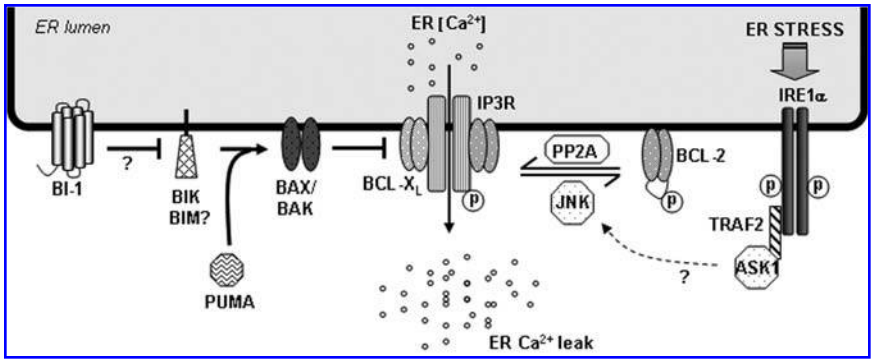

FIG. 3. Regulation of ER calcium homeostasis by the BCL-2 protein family. Different anti- and proapoptotic members of the BCL-2 family of proteins are located at the ER membrane, where they have an important role regulating ER calcium content. BCL-2 and BCL-X_L interact with the IP3R calcium channel, modulating its activity. BCL-2 has been shown to increase ER calcium leak through the IP3R because of an increase on its phosphorylation levels. BAX and BAK have the opposite effect on ER calcium content, a function that may be further modulated by BH3-only proteins (such as PUMA and BIK). 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. Alternatively, ER stress activates the IRE1α/JNK pathway that may alter the activity of BCL-2 at the ER membrane. BI-1 is also located at the ER membrane, where it regulates calcium homeostasis.

Two BH3-only proteins, PUMA and NOXA, have been recently shown to be strongly induced 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 members of the BCL-2 family to be upregulated by ER stress (80). A broad RNA-interfering screening for genes that regulate ER stress-mediated apoptosis corroborated the functional role of PUMA on the process and additionally identified NOXA as part of the pathway (20). These data are further supported by the fact that *puma*- or *noxa*-deficient murine embryonic fibroblasts (MEFs) are partially resistant to apoptosis induced by ER injuries, probably having complementary roles (56). In this line, upregulation of PUMA and NOXA under ER-stress conditions may result on the activation of the apoptosis through an interaction with a BCL-2 family member at the mitochondria. PUMA interacts with BAX, modulating ER calcium homeostasis (60). Furthermore, inhibition of PUMA activity by the small chaperone p23 may also occur at the ER (78).

Another BH3-only member, BIK, is primarily localized to the ER (21). Once activated, BIK appears capable of influencing ER calcium release. BAK becomes oligomerized at the ER after BIK expression, and BIK requires BAX/BAK to trigger calcium release and apoptosis (62). Another interesting example of ER-linked BH3-only members is BIM. Under normal conditions, BIM is found in the dynein motor complex of the microtubule cytoskeleton, whereas BIM has been reported to translocate to the ER after ER stress induction, where it may promote caspases activation (64). In summary, different BH3-only members have particular mechanisms involved in apoptosis, where some members are (a) induced at the transcriptional level, (b) their intracellular localization is altered because of posttranslational modifications, or (c) their protein-binding properties are modified.

Another protein, identified as BAX inhibitor-1 (BI-1), is functionally related to the BCL-2 family of proteins and is primarily located in the ER membrane (105). BI-1 is an anti-apoptotic protein that partially affects ER stress–dependent cell death (11). BI-1 has six transmembrane regions and no obvious homology with BCL-2–related proteins, yet it interacts with different members of the family such as BCL-X_L and BCL-2 (11, 105). BI-1 expression alters calcium homeostasis, in which its expression reduces ER calcium content (11, 103). The exact mechanism by which BI-1 modulates cell death downstream of ER stress remains elusive.

BEYOND APOPTOSIS: THE BCL-2 PROTEIN FAMILY AS "ER STRESS SENTINELS"

In the next part of this review, I discuss recent data supporting new functions of the BCL-2 protein family at the ER, controlling ER membrane remodeling and the UPR.

The unfolded protein response

The UPR is an elegant signaling pathway that aims the adaptation to ER stress conditions. The UPR was first characterized in yeast, in which a single signaling pathway governs the response to ER stress, which is mediated by a type I transmembrane ER protein known as IRE1 (inositol-requiring transmembrane kinase/endonuclease) (13, 20, 91, 93). In higher eukaryotes, the UPR is complex pathway mediated at least by three distinct UPR signaling pathways initiated by the sensors IRE1α, PERK (PKR-like ER kinase), and ATF6 (activating transcription factor 6). These proteins transduce adaptive signals to the cytosol and nucleus, leading to global changes on the function of the ER (30).

IRE1α is the most evolutionarily conserved pathway of the UPR. Nevertheless, little is known about the regulation of IRE1α activity. IRE1α is a Ser/Thr protein kinase and endoribonuclease that, on activation, initiates the unconventional splicing of the mRNA encoding the transcriptional factor X-Box-binding protein 1 (XBP-1) (9, 51, 108). In mammalian cells, a 26-nucleotide intron of *xbp-1* mRNA is spliced out by activated IRE1α, leading to a shift in the coding reading frame. This splicing event promotes the expression of a more stable and potent transcriptional activator that controls the upregulation of a broad spectrum of UPR-related genes involved in protein folding, redox metabolism, ER-associated degradation, and protein quality control (50).

One of the key findings in the UPR field was the discovery of the possible mechanism underlying IRE1α activation. It is proposed that, under normal conditions, the chaperone BiP binds to IRE1α through its ER luminal domain, maintaining the protein in an inactive monomeric state (5, 40). In ER stressed cells, BiP is preferentially bound to misfolded proteins, thereby releasing IRE1α to multimerize and autophosphorylate its cytosolic domain. This phosphorylation event triggers the activation of the RNase activity, initiating *xbp-1* mRNA splicing and UPR responses. This model suggests a direct mechanism by which IRE1α senses the accumulation of misfolded proteins at the ER lumen. However, the role of BiP in regulating IRE1α activation has been questioned, because mutations in the BiP binding site of IRE1α do not alter its activation (41). Recently, new insights into the mechanism of IRE1α activation were proposed when Peter Walter and Randall Kaufman's groups independently solved the structure on the ER luminal domain of yeast and human IRE1. Walter's group speculated that misfolded proteins may directly bind to the N-terminal region of IRE1, facilitating its oligomerization through a binding motif similar to the MHC-like groove (14, 112). The cytosolic domain of activated IRE1α binds to the adaptor protein TRAF2 (TNF-associated factor 2), triggering the activation of the apoptosis signal–regulating kinase 1 (ASK1) and JNK pathway (67, 98) and the NF-κB pathway (33). Activation of the ER-resident caspase-12 has been suggested to be linked the UPR pathway through an interaction with TRAF2 and possibly with active IRE1α (106), but a complex between procaspase-12/TRAF2/IRE1α has not been described. In addition, the contribution of caspase-12 to ER stress–mediated apoptosis is actively debated (85, 89). However, calpain activation through ER calcium release has been suggested to contribute to capsase-12 and JNK activation with proapoptotic effects (65). As I discuss later, we recently described a molecular link between the BCL-2 protein family and the activation process of IRE1α.

Some UPR effects are mediated by PERK. Activated PERK inhibits the translation of proteins into the ER, alleviating the

stress in this organelle by decreasing the overload of misfolded proteins through the inactivation of the initiation factor eIF2α (86). PERK controls the expression of ATF4 (activating transcription factor 4) (86). ATF4 is a transcription factor that induces the expression of UPR genes that function in amino acid and redox metabolism, including *chop/gadd153* and *gadd34* (61, 94, 115). A second UPR pathway is initiated by ATF6, a type II ER transmembrane protein encoding a bZIP transcriptional factor on its cytosolic domain (25, 109). On ER stress induction, ATF6 is processed at the Golgi, releasing its cytoplasmic domain, which acts as a transcriptional activator. Cleaved ATF6 may increase the expression of some ER chaperones and *xbp-1* transcription (51), but the role of ATF6 on the UPR is still controversial (50).

Involvement of the BCL-2 protein family in the UPR

Recently the possible function of the BCL-2 protein family on ER stress responses was addressed. BAX and BAK were shown to regulate the UPR through a modulation of the amplitude of IRE1α signaling, without affecting PERK activity (26) (Fig. 4). BAX and BAK DKO cells displayed a specific deficiency in the autophosphorylation and oligomerization of IRE1α. As a consequence, BAX/BAK-deficient cells showed decreased expression of IRE1α-downstream signals, including JNK phosphorylation and XBP-1 splicing. More important, when a BAX/BAK-inducible DKO mouse model was challenged with the ER stress–inducing drug tunicamycin (inhibits *N*-glycosylation), a failure to handle ER stress in the liver was observed, as revealed by impairment of IRE1α signaling in this organ and extensive tissue damage (26).

At the biochemical level, BAX and BAK form a protein complex with the cytosolic domain of IRE1α, where its enzymatic activities are located. This interaction was also recapitulated with recombinant proteins, suggesting direct binding. The association between BAX and IRE1α is increased in cells undergoing ER stress, and their physical association depends on the presence of the conserved BH3 and BH1 domains. At the mechanistic level, it was proposed that BAX and BAK may fa-

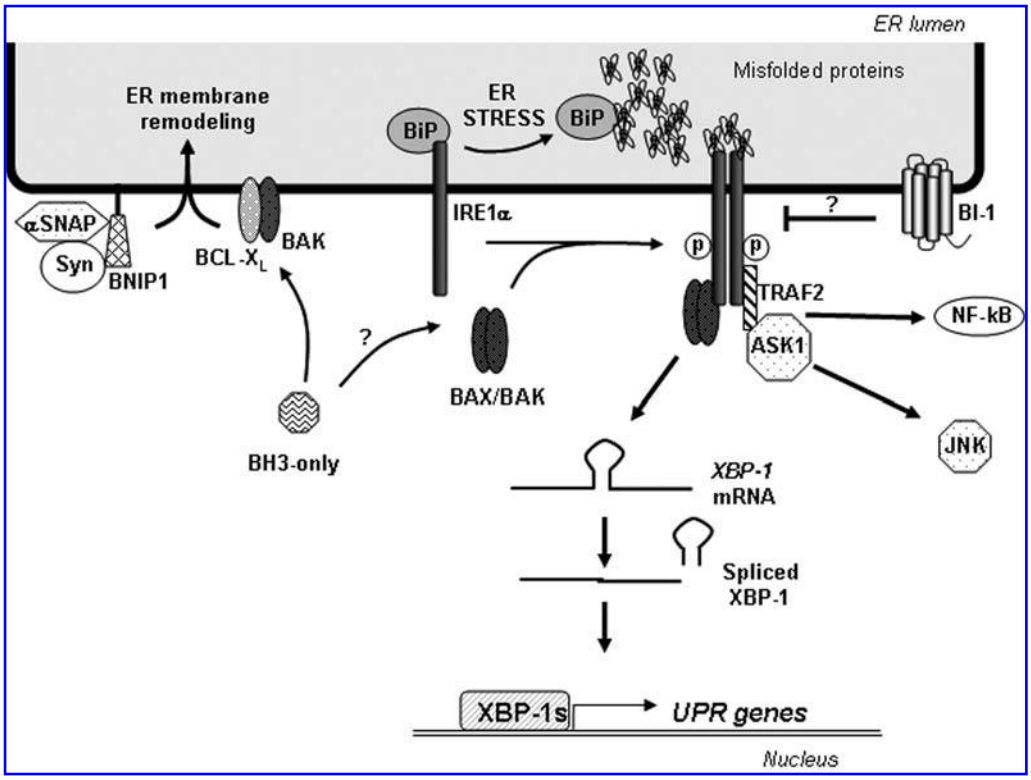

FIG. 4. Regulation of the UPR and ER membrane remodeling by the BCL-2 family of proteins. *Regulation of the UPR:* Under nonstress conditions, IRE1α is maintained in an inactive monomeric state through its binding to the ER chaperone BiP. During a stress response, BiP binds preferentially to unfolded/misfolded proteins in the ER lumen, releasing its inhibitory effect over IRE1α. Then IRE1α dimerizes and autophosphorylates, leading to its activation. Phosphorylation of IRE1α triggers its endoribonuclease activity, which mediates the processing of the mRNA encoding *XBP-1*. Alternatively, activated IRE1α interacts with the adaptor protein TRAF2, leading to the activation of the JNK and NF-κB pathways. Activation of IRE1α requires the binding of accessory proteins BAX and BAK, which may stabilize the active form of IRE1α, fomenting its prosurvival 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_L at the ER membrane leads to drastic changes on the ER structure. These effects may be modulated by some BH3-only proteins and the ryanodine receptor. The BH3-only protein BNIP1 forms a protein complex with syntaxin-18 (Syn) and α-SNARE, regulating ER-membrane remodeling and ER-structure patterning.

cilitate the activation of IRE1α by stabilizing its active form through the formation of a stable protein complex (26) (see Fig. 4). Overall, these findings suggest a new and unexpected role for BAX and BAK, in which they act as accessory proteins for the activation of certain UPR prosurvival signaling pathways. This activity at the ER contrasts completely with the known proapoptotic function of BAX and BAK at the mitochondria.

Many questions remain open for further study and are summarized as follows: Is there a connection between the regulation of calcium physiology by the BCL-2 family of proteins and the UPR? Is the modulation of IRE1α signaling by BAX and BAK tissue or signal specific? Do BAX and BAK affect the activation of IRE1α under physiologic conditions (*i.e.*, B-cell activation (35, 81) or in insulin-secreting pancreatic beta cells (49)]? Is the BCL-2 protein family important for the transition from an adaptive state to the activation of cell death under irreversible ER damage?

BAX-inhibitor 1: the evolutionary link between the UPR and the BCL-2 protein family?

The laboratory of John Reed recently described the ER stress responses on BI-1–knockout mice. BI-1–deficient mice showed hyperactivation of the IRE1α pathway *in vivo* in a model of hepatic and renal ischemia (3), an experimental model known to trigger ER stress. Their results suggest that BI-1 has an inhibitory activity on IRE1α, contrasting with the opposite effect of BAX and BAK on the pathway (see Fig. 4). The authors speculated that this phenotype may be due to an impaired IRE1α activation, but the mechanism underlying their observations was not addressed. The effects of BI-1 on the UPR were specific because the PERK pathway was not affected in BI-1–deficient mice (3). Because of this, it is possible to speculate that BI-1 has an additional activity outside of the regulation of apoptosis at the ER, where it negatively controls IRE1α activation. BI-1 was cloned in yeast, searching for inhibitors of BAX lethality after overexpression. These studies reveled that BI-1 is well conserved in yeast, plants, and many other organisms (10, 34). Overexpression of BI-1 in yeast gives protection against heat shock and exposure to H_2O_2. However, the biologic function of BI-1 in yeast is not known, and its role on the UPR has not been explored yet.

We envision a model in which a complex protein platform operates at the ER membrane to control IRE1α signaling. It remains to be determined whether different BH3-only proteins modulate the association of BAX and BAK with IRE1α. BH3-only proteins may compete for the BH1/BH3 domain binding site on IRE1α, constituting possible negative-feedback-loop regulation. I predict that disruption of the interaction between BAX/BAK and IRE1α may shut down the prosurvival effects of this pathway, sensitizing cells to apoptosis. Overall, this model proposes that the balance between anti- and proapoptotic members at the ER membrane may determine the ability of a cell to respond to ER stress by controlling the amplitude of the UPR signaling.

A role of the BCL-2 protein family on ER membrane remodeling

Recent reports suggest that the expression of some proapoptotic members of the BCL-2 family of proteins alter the structure of the ER. BAK was recently shown to regulate ER swelling and the remodeling of the reticular structure (42). Coexpression of BAK and BCL-X_L or BAK mutants in the BH3 domain provoked extensive swelling and vacuolization of ER cisternae. These effects were specific to the location of BAK in the ER and not the mitochondria. Interestingly, the coexpression of upstream BH3-only activators (*i.e.*, BIM or truncated BID) with BCL-X_L recapitulated ER swelling and vacuolization under conditions in which the ryanodine receptor–calcium channel was inhibited (42). Surprisingly, the effects of BH3-only proteins on ER swelling were proposed to be specifically dependent on BAK expression and not BAX, the closest homologue.

The BH3-only member BNip1 was recently described as a component of a protein complex comprising syntaxin 18, an ER-located soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) (66). At the functional level, BNip1 was shown to participate in the formation and modeling of the ER-network structure, but not in membrane trafficking between the ER and Golgi. In addition, the BH3 domain was shown to be important for the binding of BNip1 to α-SNAP, an adaptor that serves as a link between the chaperone ATPase NSF and SNAREs (66). These results unmasked possible cross-talk between apparently independent cellular events, apoptosis, and ER membrane fusion. In summary, different components of the BCL-2 family of proteins have been shown to play important roles in ER physiology, and these effects are mediated through the conserved BH domains. Alternatively, BAX and BAX have been recently shown to regulate mitochondria morphogenesis (38), suggesting that these proteins may have broad regulatory effects on organelle morphogenesis.

INTERPLAY BETWEEN THE UPR, AUTOPHAGY, AND THE BCL-2 PROTEIN FAMILY

Autophagy refers to the global process by which intracellular components are recycled through lysosome degradation (review in 54). It has a critical survival role 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 most studied form of autophagy is macroautophagy, hereafter referred to as autophagy. The generation of knockout mice for autophagic-related genes has shown that autophagy participates in diverse processes, including development, cell differentiation, tissue remodeling, immunity, host-to-pathogen response, and cell death/survival under stress conditions (54). The hallmark of autophagy is the formation of double-membrane–bound 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 autophagy-related genes (termed *atg* genes). Atg proteins have different functions in the process,

including the formation of a protein kinase–autophagy regulatory complex that responds to upstream signals, (*i.e.*, nutrient limitation), a lipid kinase signaling complex involved in vesicle nucleation, a ubiquitin-like protein conjugation pathway required for vesicle expansion and completion, and a retrieval pathway for catalyzing the disassembly of Atg protein complexes from matured autophagosomes (55). LC3 (also known as Atg8) is the only known marker that specifically localizes to autophagosomes (36). In addition, recent studies indicate that autophagy has a crucial role in the continuous turnover of misfolded protein content in neurons, acting as an alternative protein quality control (review in 15, 82). This concept was demonstrated recently when the disruption of autophagy in neurons was performed by using inducible knockout mouse models. Inhibition of autophagy in the central nervous system resulted in spontaneous neurodegeneration due to the accumulation of ubiquinated and misfolded proteins (24, 43).

Beclin-1 (also known as Atg6) was the first identified mammalian autophagy gene product (1). Beclin-1 is a haploinsufficient tumor suppressor that was originally isolated as a BCL-2–interacting protein (57, 75, 110). More interestingly, BCL-2 was recently shown negatively to regulate Beclin-1–dependent autophagy through direct binding (73) (see Fig. 4). Beclin-1 mutants that cannot bind to BCL-2 induce more autophagy than does wild-type Beclin-1. Surprisingly, this regulatory activity of BCL-2 on autophagy was specifically attributed to the expression of this protein at the ER membrane, suggesting that signaling events originated from the ER may have an important role in the process.

Autophagy is enhanced in conditions in which apoptosis is inhibited (*i.e.*, BAX and BAK deficiency or BCL-X_L overexpression) (92), suggesting a constant interplay between apoptosis and autophagy-related pathways. A recent report indicates that ER stress triggered by the expression of mutant proteins linked to Huntington disease induces autophagy (46, 63). These effects were shown to be controlled in part by the PERK/eIF2α pathway. At the molecular level, it was proposed that PERK increases autophagy through the upregulation of Atg12 (45) (Fig. 5). Thus, autophagy is a cellular defense mechanism against misfolded proteins that is tightly regulated by the UPR to enhance the clearance of protein aggregates. Another report confirmed this model by showing a direct activation of autophagy by ER stress (70). The authors increased the complexity of the pathway by showing that the formation of LC3-contain-

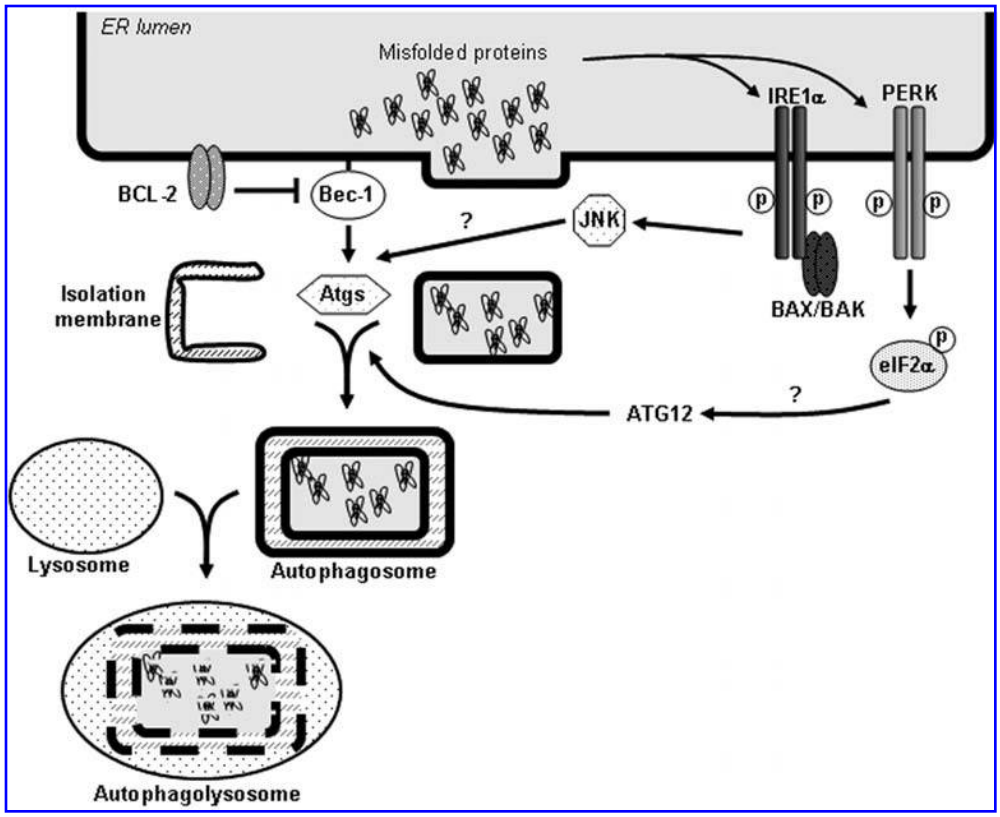

FIG. 5. A role of ER stress and the BCL-2 protein family on autophagy. Accumulation of misfolded protein at the ER lumen triggers autophagy, which may lead to a decreased intracellular protein aggregation through lysosome degradation. The hallmark of autophagy is the formation of double-membrane–bound autophagosomes from "isolation membranes" that engulf intracellular contents (*i.e.*, damaged organelles and cytosol). Autophagosomes fuse with lysosomes to form autophagolysosomes. Autophagy is a highly regulated process and is mediated by different ATG proteins. A member of this family, Beclin-1 (Bec-1), is negatively regulated through an interaction with BCL-2 at the ER membrane. Conversely, activation of IRE1α may increase the levels of autophagy through the activation of JNK, whereas activated PERK increases autophagy though the inhibition of the initiation factor eIF2α by phosphorylation and the subsequent upregulation of ATG12.

ing autophagosomes was dependent on the activation of the IRE1α pathway and not PERK. Surprisingly, it was suggested that IRE1α regulates autophagy through its kinase activity and by the activation of the TRAF2/JNK pathway and not the RNAse activity (70). It remains to be determined whether the regulatory activity of the BCL-2 family of proteins (*i.e.*, BAX and BAK) on IRE1α affects autophagy (*i.e.*, modulation of Beclin-1/BCL-2).

The relation between ER stress and autophagy is an evolutionary conserved process, because a recent report demonstrated that ER stress stimulates the assembly of the pre-autophagosomal structure in yeast (107). The authors speculated that IRE1 pathway, the only ER stress sensor present in yeast, may be responsible for the activation of autophagy by ER stress. In addition, Peter Walter's group recently proposed a role for autophagy in controlling the rate of ER expansion during yeast UPR.

CONCLUSIONS AND THERAPEUTIC PERSPECTIVES

I have summarized different pieces of evidence suggesting that the BCL-2 family of proteins has evolved to regulate multiple processes involved in cell survival under stress conditions. The global view of the current state of the field indicates that the BCL-2–related proteins are not only the "death gateway" keeper (as upstream regulators of caspases), but they also have multiple functions in essential processes for the cell. BCL-2–related proteins are particularly important in the physiologic maintenance of the ER, where they operate as (a) a calcium rheostat, (b) modulators of the UPR, (c) regulators of ER network structure, and (d) regulators of autophagy. In addition, examples of a role of the BCL-2 family of proteins in cell-cycle regulation (87, 113), DNA damage responses (37, 114), and glucose/energy metabolism (16) are available, strongly supporting the notion that the BCL-2 protein family is a multifunctional group of proteins that, under normal conditions, participate in essential cellular process. In doing so, the BCL-2 protein family may represent specialized stress sentinels that actively participate in essential processes, allowing a constant homeostatic "quality control." In response to irreversible cellular damage, particular BCL-2 family members may turn into direct activators of apoptosis.

Mutations in specific genes are responsible for a variety of neurologic disorders due to the misfolding and accumulation of abnormal protein aggregates in the brain. In many of these diseases, it has been suggested that alteration in the homeostasis of the ER contributes significantly to neuronal dysfunction. These diseases include Parkinson's disease (32, 84), Alzheimer's disease (22), prion diseases (27, 28, 31), amyotrophic lateral sclerosis (ALS) (97), Huntington's disease (63, 90) and many others (see list of diseases in 86). Consequently, the first steps in the death pathways downstream of ER stress represent important therapeutic targets. In this line of thinking, pharmacologic manipulation of the activity of the BCL-2 protein family may have beneficial consequences to treat these fatal diseases. Different small molecules and synthetic peptides are currently available with proven therapeutic applications in mouse disease models, including BCL-2 inhibitors (71), BAX channel inhibitors (29), BAX/BAK activator peptides (100, 101) and many others (see reviews in 52, 79). These drugs may be used as pharmacologic tools to manipulate the activity of stress-signaling pathways regulated by the BCL-2 protein family (*i.e.*, autophagy, calcium metabolism, or the UPR) and their possible role in pathologic conditions.

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ABBREVIATIONS

ASK1, Apoptosis signal-regulating kinase 1; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; Atg, autophagy-related genes; BH, BCL-2 homology; DKO, double knockout; ER, endoplasmic reticulum; IP3R, inositol triphosphate receptor; IRE1, inositol-requiring transmembrane kinase/endonuclease; JNK, c-Jun N-terminal kinase; MEFs, murine embryonic fibroblasts; PERK, PKR-like ER kinase; SNARE, ER-located soluble *N*-ethylmaleimide–sensitive factor attachment receptor; TRAF2, TNF-associated factor 2; UPR, unfolded protein response; XBP-1, X-Box–binding protein 1.

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