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Signaling cell death from the endoplasmic reticulum stress response

Gordon C Shore¹, Feroz R Papa² and Scott A Oakes³

Inability to meet protein folding demands within the endoplasmic reticulum (ER) activates the unfolded protein response (UPR), a signaling pathway with both adaptive and apoptotic outputs. While some secretory cell types have a remarkable ability to increase protein folding capacity, their upper limits can be reached when pathological conditions overwhelm the fidelity and/or output of the secretory pathway. Irremediable 'ER stress' induces apoptosis and contributes to cell loss in several common human diseases, including type 2 diabetes and neurodegeneration. Researchers have begun to elucidate the molecular switches that determine when ER stress is too great to repair and the signals that are then sent from the UPR to execute the cell.

Addresses

¹ Department of Biochemistry and Goodman Cancer Center, McGill University, Montreal, Quebec, H3G 1Y6, Canada

² Department of Medicine, Diabetes Center, & California Institute for Quantitative Biosciences, University of California-San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA

³ Department of Pathology, University of California-San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA

Corresponding authors: Shore, Gordon C (gordon.shore@mcgill.ca), Oakes, Scott A (scott.oakes@ucsf.edu)

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Introduction

The lumen of the endoplasmic reticulum (ER) is a unique cellular environment optimized to carry out the three primary tasks of this organelle: calcium storage and release, protein folding and secretion, and lipid biogenesis [1]. A range of cellular disturbances lead to accumulation of misfolded proteins in the ER, including point mutations in secreted proteins that disrupt their proper folding, sustained secretory demands on endocrine cells, viral infection with ER overload of virus-encoding protein, and loss of calcium homeostasis with detrimental effects on ER-resident calcium-dependent chaperones [2^{**},3,4]. The tripartite unfolded protein response (UPR) consists of three ER transmembrane proteins

(IRE1 α , PERK, ATF6) that alert the cell to the presence of misfolded proteins in the ER and attempt to restore homeostasis in this organelle by increasing ER biogenesis, decreasing the influx of new proteins into the ER, promoting the transport of damaged proteins from the ER to the cytosol for degradation, and upregulating protein folding chaperones [5]. The adaptive responses of the UPR can markedly expand the protein folding capacity of the cell and restore ER homeostasis [6]. However, if these adaptive outputs fail to compensate because ER stress is excessive or prolonged, the UPR induces cell death. The cell death pathways collectively triggered by the UPR include both caspase-dependent apoptosis and caspase-independent necrosis. While many details remain unknown, we are beginning to understand how cells determine when ER stress is beyond repair and communicate this information to the cell death machinery. For the purposes of this review, we focus on the apoptotic outputs triggered by the UPR under irremediable ER stress. While the ER contains numerous additional signaling platforms and targets that respond to diverse apoptotic stimuli (e.g. those associated with the Bap31 complex [7,8]), their formal link to UPR-driven apoptosis remains to be determined.

The proximal unfolded protein response sensors

UPR signaling is initiated by three ER transmembrane proteins: IRE1 α , PERK, and ATF6. The most ancient ER stress sensor, IRE1 α , contains an ER luminal domain, a cytosolic kinase domain, and a cytosolic RNase domain [9,10]. In the presence of unfolded proteins, IRE1 α 's ER luminal domains homo-oligomerize, leading first to kinase trans-autophosphorylation and subsequent RNase activation. Dissociation of the ER chaperone BiP from IRE1 α 's luminal domain in order to engage unfolded proteins may facilitate IRE1 α oligomerization [11]; alternatively, the luminal domain may bind unfolded proteins directly [12]. PERK's ER luminal domain is thought to be activated similarly [13,14]. The ATF6 activation mechanism is less clear. Under ER stress, ATF6 translocates to the Golgi and is cleaved by Site-1 and Site-2 proteases to generate the ATF6(N) transcription factor [15].

All three UPR sensors have outputs that attempt to tilt protein folding demand and capacity back into homeostasis. PERK contains a cytosolic kinase that phosphorylates eukaryotic translation initiation factor 2 α (eIF2 α), which impedes translation initiation to reduce the protein

load on the ER [16]. IRE1 α splices *XBP1* mRNA, to produce the homeostatic transcription factor XBP1s [17,18]. Together with ATF6(N), XBP1s increases transcription of genes that augment ER size and function [19]. When eIF2 α is phosphorylated, the translation of the activating transcription factor-4 (ATF4) is actively promoted and leads to the transcription of many pro-survival genes [20]. Together, these transcriptional events act as homeostatic feedback loops to reduce ER stress. If successful in reducing the amount of unfolded proteins, the UPR attenuates.

However, when these adaptive responses prove insufficient, the UPR switches into an alternate mode that promotes apoptosis. Under irremediable ER stress, PERK signaling can induce ATF4-dependent upregulation of the CHOP/GADD153 transcription factor, which inhibits expression of the gene encoding anti-apoptotic BCL-2 while upregulating the expression of oxidase ERO1 α to induce damaging ER oxidation [21,22]. Sustained IRE1 α oligomerization leads to activation of apoptosis signal-regulating kinase 1 (ASK1) and its downstream target c-Jun NH₂-terminal kinase (JNK) [23,24]. Phosphorylation by JNK has been reported to both activate pro-apoptotic BIM and inhibit anti-apoptotic BCL-2 (see below). Small-molecule modulators of ASK1 have been shown to protect cultured cells against ER stress-induced apoptosis, emphasizing the importance of the IRE1 α -ASK1-JNK output as a death signal in this pathway [25^{*}]. In response to sustained oligomerization, the IRE1 α RNase also causes endonucleolytic decay of hundreds of ER-localized mRNAs [26^{**}]. By depleting ER cargo and protein folding components, IRE1 α -mediated mRNA decay may worsen ER stress, and could be a key aspect of IRE1 α 's pro-apoptotic program [27^{**}]. Recently, inhibitors of IRE1 α 's kinase pocket have been shown to conformationally activate its adjacent RNase domain in a manner that enforces homeostatic XBP1s without causing destructive mRNA decay [27^{**}], a potentially exciting strategy for preventing ER stress-induced cell loss.

When deciding whether to switch into apoptotic mode, cells might use one or more 'timers' to indicate if UPR signaling remains continuously active under high or chronic ER stress. For example, sustained PERK activity could result in protracted translation attenuation, which should be incompatible with survival, as well as high levels of pro-apoptotic CHOP. Similarly, high-level mRNA degradation mediated by IRE1 α may deplete ER protein folding capacity further, and along with JNK signaling push the cell towards apoptosis. Thus, the continuous activation of the proximal sensors IRE1 α and PERK may constitute a 'timer' that triggers the switch to apoptosis under irremediable ER stress. Moreover, the ultimate response may depend on cell context. For example, the ability of IRE1 α to complex with

regulators such as BAX/BAK and Bax Inhibitor 1 (BI-1) at the ER may influence its ability to remediate ER stress and/or potentially signal apoptosis [28,29^{*}].

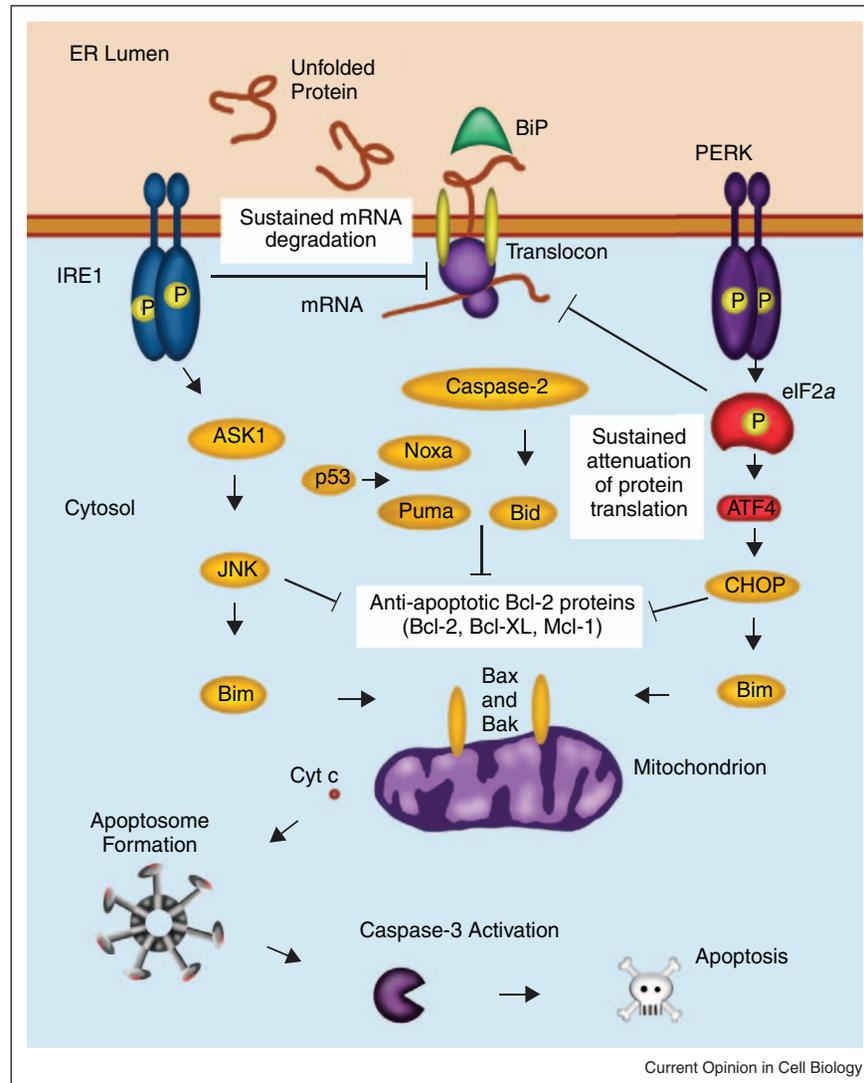
The BCL-2 family and the mitochondrial apoptotic pathway

A wealth of genetic and biochemical data argues that the intrinsic (mitochondrial) apoptotic pathway is the major cell death pathway induced by the UPR, at least in most cell types. This apoptotic pathway is set in motion when several toxic proteins (e.g. cytochrome *c*, Smac/Diablo) are released from mitochondria into the cytosol where they lead to activation of downstream effector caspases (e.g. caspase-3) [30]. The BCL-2 family, a large class of both pro- and anti-survival proteins, tightly regulates the intrinsic apoptotic pathway by controlling the integrity of the outer mitochondrial membrane [31]. This pathway is set in motion when cell injury leads to the transcriptional and/or post-translational activation of one or more BH3-only proteins, a structurally diverse class of pro-apoptotic BCL-2 proteins that share sequence similarity only in a short alpha helix (~9–12 a.a.) known as the BCL-2 homology 3 (BH3) domain [32]. Once activated, BH3-only proteins lead to loss of mitochondrial integrity by disabling mitochondrial protecting proteins (e.g. BCL-2, BCL-XL, MCL-1) and (for a subset) directly triggering the oligomerization of the multi-domain pro-apoptotic BAX and BAK proteins that drive the permeabilization of the outer mitochondrial membrane (Figure 1).

ER stress has been reported to activate at least four distinct BH3-only proteins (BID, BIM, NOXA, PUMA) that then signal the mitochondrial apoptotic machinery (i.e. BAX/BAK) [33^{*},34^{*},35^{*}]. Each of these BH3-only proteins is activated by ER stress in a unique way. For example, BID is proteolytically cleaved at a caspase recognition site into a potent apoptotic signal [33^{*}]. The *Bim* gene is transcriptionally upregulated and its protein product stabilized through dephosphorylation in response to ER stress [34^{*}]. Cells individually deficient in any of these BH3-only proteins are modestly protected against ER stress-inducing agents, but not nearly as resistant as cells null for their common downstream targets BAX and BAK [36]—the essential gatekeepers to the mitochondrial apoptotic pathway. Moreover, cells genetically deficient in both *Bim* and *Puma* are more protected against ER stress-induced apoptosis than *Bim* or *Puma* single knockout cells [37], arguing that several BH3-only proteins are necessary for efficient activation of BAX/BAK-dependent apoptosis under conditions of irremediable ER stress.

The ER stress sensor that signals these BH3-only proteins is known in a few cases (i.e. BIM is downstream of PERK); however, we do not yet understand how the UPR communicates with most of the BH3-only proteins.

Figure 1



Connections from the UPR to the mitochondrial apoptotic pathway. Under excessive ER stress, the ER transmembrane sensors IRE1 α and PERK send signals through the BCL-2 family of proteins to activate the mitochondrial apoptotic pathway. In response to unfolded proteins, IRE1 α oligomerizes and induces endonucleolytic decay of hundreds of ER-localized mRNAs, depleting ER protein folding components and leading to worsening ER stress. Phosphorylated IRE1 α also recruits TNF receptor-associated factor 2 (TRAF2) and activates apoptosis signaling kinase 1 (ASK1) and its downstream target c-Jun NH₂-terminal kinase (JNK). JNK then activates pro-apoptotic BIM and inhibits anti-apoptotic BCL-2. These conditions result in dimerization of PERK and activation of its kinase domain to phosphorylate eukaryotic translation initiation factor 2 α (eIF2 α), which causes selective translation of activating transcription factor-4 (ATF4). ATF4 upregulates expression of the CHOP/GADD153 transcription factor, which inhibits the gene encoding anti-apoptotic BCL-2 while inducing expression of pro-apoptotic BIM. ER stress also promotes p53-dependent transcriptional upregulation of Noxa and Puma, two additional pro-apoptotic BH3-only proteins. Furthermore, high levels of UPR signaling induce initiator caspase-2 to proteolytically cleave and activate pro-apoptotic BID upstream of the mitochondrion. In addition to antagonizing pro-survival BCL-2 members, cleaved BID, BIM and PUMA activate Bax and/or Bak. Hence, in response to excessive UPR signaling, the balance of BCL-2 family proteins shifts in the direction of apoptosis and leads to the oligomerization of BAX and BAK, two multi-domain pro-apoptotic BCL-2 family proteins that then drive the permeabilization of the outer mitochondrial membrane, apoptosome formation, and activation of executioner caspases such as caspase-3. Figure adapted with permission from the *Journal of Cell Science* [58].

Moreover, it is not known if all of the above BH3-only proteins are simultaneously set in motion by all forms of ER stress or if a subset is activated under specific pathological stimuli that injure this organelle. Understanding the molecular details of how ER damage is communicated to the mitochondrial apoptotic machinery is critical

if we want to target disease-specific apoptotic signals sent from the ER.

Initiator and executor caspases

Caspases, or cysteine-dependent aspartate-directed proteases, play essential roles in both initiating apoptotic

signaling (initiator caspases-2, 4, 8, 12) and executing the final stages of cell demise (executioner caspases-3, 7, 9) [38]. The executioner caspases are proteolytically activated through either mitochondrial-dependent apoptosome formation or death receptor activation of upstream initiator caspases (i.e. caspase-8, 10). Given the prominent role of the mitochondrial apoptotic pathway in ER stress-induced death, it is not surprising that the executioner caspases (caspase-3, 7, 9) are critical for cell death resulting from damage to this organelle. On the contrary, there has been much controversy regarding the role of initiator caspases in ER stress-induced apoptosis. Caspase-12 was the first caspase reported to localize to the ER and become activated by UPR signaling in murine cells [39]. However, caspase-12 was subsequently shown to be downstream of BAX/BAK-dependent mitochondrial permeabilization and executioner caspase activation in this pathway [40], arguing that its role is probably limited to amplifying rather than initiating ER stress-induced apoptosis. Moreover, most humans fail to express a functional CASP12 due to a polymorphism that creates a nonsense mutation in the coding region [41], which rules out an essential role for this protease in human ER stress signaling. More recently, caspase-2 was found to be the premitochondrial protease that proteolytically cleaves and activates the BH3-only protein BID in response to ER stress [33]. Genetic knockdown or pharmacological inhibition of caspase-2 confers resistance to ER stress-induced apoptosis [42]. How the UPR activates caspase-2 and whether other initiator caspases, such as caspase-4, are also involved remains to be determined.

Calcium and cell death

Although an extreme depletion of ER luminal Ca^{2+} concentrations is a well-documented initiator of the UPR and ER stress-induced apoptosis or necrosis, it represents a relatively non-physiological stimulus. Given that Ca^{2+} signaling from the ER is likely coupled to most pathways leading to apoptosis, however, it is not surprising that this also extends to UPR overload. For example, recent evidence in macrophages indicates that UPR-induced activation of ERO1- α via CHOP results in stimulation of inositol 1,4,5-triphosphate receptor (IP3R) [43], the major release channel for luminal Ca^{2+} from the ER. Although pathways may exist for ER Ca^{2+} release independently of IP3 receptors, many seemingly disparate pathways appear to converge on the IP3R platform. Consistent with this, all three sub-groups of the BCL-2 family at the ER regulate IP3R activity. Mechanistically, this might ultimately result from titrations of pro-survival BCL-X_L, BCL-2, and MCL-1 that physically associate with IP3R [44]. Release of ER Ca^{2+} via IP3R into the cytoplasm could of course influence multiple pathways upstream of the core apoptosis machinery. However, a significant fraction of IP3R is a constituent of highly specialized tethers that physically attach ER cisternae to mitochondria (mitochondrial-

associated membrane) and regulate local Ca^{2+} dynamics at the ER-mitochondrion interface [45,46]. This results in propagation of privileged IP3R-mediated Ca^{2+} oscillations into mitochondria, which can influence cell survival in multiple ways. In an extreme scenario, massive transmission of Ca^{2+} into mitochondria results in Ca^{2+} overload and cell death by caspase-dependent and caspase-independent means [46], particularly via the pathway involving the permeability transition pore/cyclophilin D complex [47]. More refined transmission regulated by the BCL-2 axis at the ER can influence cristae junctions and the availability of cytochrome *c* for its release across the outer mitochondrial membrane [48]. Finally, such regulated Ca^{2+} transmission to mitochondria is a key determinant of mitochondrial bioenergetics, which is linked not only to potential apoptotic responses, but importantly to survival/death mechanisms dependent on macroautophagy [49].

ER stress-induced cell loss and disease

Mounting evidence suggests that ER stress-induced apoptosis contributes to a range of human diseases of cell loss, including diabetes, neurodegeneration, stroke, and heart disease, to name a few (reviewed in REF [50]). The cause of ER stress in these distinct diseases varies depending on the cell type affected and the intracellular and/or extracellular conditions that disrupt proteostasis. For example, some cases of inherited amyotrophic lateral sclerosis (ALS) are caused by toxic, gain-of-function point mutations in superoxide dismutase-1 (SOD1). Other neurodegenerative diseases, such as Huntington, result from mutant proteins (e.g. huntingtin) containing expanded glutamate repeat sequences. Both mutant SOD1 and mutant huntingtin proteins aggregate, exhaust proteasome activity, and result in secondary accumulations of misfolded proteins in the ER [51,52]. In the early stages of type 2 diabetes, peripheral insulin resistance challenges pancreatic beta cells to secrete greater amounts of insulin in order to maintain euglycemia. This increased secretory demand can lead to ER stress, beta cell loss, and hyperglycemia [53]. Mutations in PERK result in massive pancreatic beta cell death and infant-onset diabetes in patients with Wolcott-Rallison syndrome [54], an autosomal recessive inherited disorder that illustrates the importance of a properly functioning UPR for beta cell health. An association between ER stress and heart disease has been implicated on a number of levels. Oxidative stress, high levels of cholesterol, and fatty acids can all cause ER stress-induced apoptosis of macrophages and endothelial cells associated with atherosclerotic plaques, leading to progression of atherosclerosis [55]. Myocardial infarction activates the UPR in cardiac myocytes; and *Askl1*^{-/-} mice show preservation of left ventricular function compared to wild-type controls after coronary artery ligation [56]. Stroke (ischemia-reperfusion injury) has also been shown to induce ER

stress-induced apoptosis, and *Chop*^{-/-} mice are partly protected from neuronal loss after stroke injury [57].

While by no means exhaustive, these examples illustrate the therapeutic potential for novel drugs that block ER stress-induced apoptosis. While chronic UPR-targeted therapies may be problematic for the many tissues that require this pathway to maintain proteostasis, acute modulation of the UPR during stroke or myocardial infarction could be an effective strategy to prevent cell loss. In the case of IRE1 α , it may be possible to use kinase inhibitors to activate its cytoprotective signaling and shut down its apoptotic outputs [27**]. Whether similar strategies will work for PERK and/or ATF6 remains to be seen. Alternatively, blocking the specific apoptotic signals that emerge from the UPR is perhaps a more straightforward strategy to prevent ER stress-induced cell loss. To this end, small molecular inhibitors of ASK and JNK are currently being tested in a variety of preclinical models of ER stress [52,53,56,57]. This is just the beginning, and much work needs to be done to validate the best drug targets in the ER stress pathway.

Conclusions

The UPR is a highly complex signaling pathway activated by ER stress that sends out both adaptive and apoptotic signals. All three transmembrane ER stress sensors (IRE1 α , PERK, ATF6) have outputs that initially decrease the load and increase the capacity of the ER secretory pathway in an effort to restore ER homeostasis. However, under extreme ER stress, continuous engagement of IRE1 α and PERK results in events that simultaneously exacerbate protein misfolding and signal death, the latter involving caspase-dependent apoptosis and caspase-independent necrosis. Advances in our molecular understanding of how these stress sensors switch from life to death signaling will hopefully lead to new strategies to prevent diseases caused by ER stress-induced cell loss.

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