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Review

Cellular response to endoplasmic reticulum stress: a matter of life or death

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Abstract

The proper functioning of the endoplasmic reticulum (ER) is critical for numerous aspects of cell physiology. Accordingly, all eukaryotes react rapidly to ER dysfunction through a set of adaptive pathways known collectively as the ER stress response (ESR). Normally, this suite of responses succeeds in restoring ER homeostasis. However, in metazoans, persistent or intense ER stress can also trigger programmed cell death, or apoptosis. ER stress and the apoptotic program coupled to it have been implicated in many important pathologies but the regulation and execution of ER stress-induced apoptosis in mammals remain incompletely understood. Here, we review what is known about the ESR in both yeast and mammals, and highlight recent findings on the mechanism and pathophysiological importance of ER stress-induced apoptosis.

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Abbreviations: BFA, brefeldin A; eIF2α, eukaryotic translation initiation factor 2 subunit α; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ESR, ER stress response; GEF, guanine nucleotide exchange factor; GLS, Golgi localization sequence; PD, Parkinson's disease; PDI1, protein disulfide isomerase 1; PP1, protein phosphatase 1; ROS, reactive oxygen species; Tg, thapsigargin; Tm, tunicamycin; uORF, upstream open reading frame; UPR, unfolded protein response; XBP-1, X-box binding protein 1

Overview: The Endoplasmic Reticulum Stress Response

The endoplasmic reticulum (ER) encompasses about half the total membrane area and one-third the newly translated proteins in a typical eukaryotic cell.[1,2] Proper ER function is therefore critical for numerous aspects of cell physiology, including vesicle trafficking, lipid and membrane biogenesis and protein targeting and secretion. Accordingly, metazoan cells react rapidly to ER dysfunction through a set of adaptive pathways known collectively as the ER stress response (ESR).[2–6]

The ESR can be triggered by disparate perturbations in normal ER function, such as the accumulation of unfolded, misfolded or excessive protein, ER lipid or glycolipid imbalances, or changes in the redox or ionic conditions of the ER lumen.[2,3,5,6] In response to such dysfunction, the ESR acts both to increase the capacity of the ER to fold and process client proteins, and to alleviate the burden on the organelle by reducing the amount of protein inside the ER. These effects are achieved through three major pathways: (I) the unfolded protein response (UPR), a transcription-dependent induction of ER lumenal chaperone proteins and other components of the secretory apparatus to augment the polypeptide folding and processing capacity of the ER;[5,7] (II) the activation of proteasome-dependent ER-associated degradation (ERAD) to remove proteins from the ER;[8–11] and (III) the control of protein translation to modulate the polypeptide traffic into the ER.[12,13] Normally, this suite of responses succeeds in restoring ER homeostasis. However, in metazoans, persistent or intense ER stress can also trigger programmed cell death, or apoptosis.[14–16] ER stress and the apoptotic program coupled to it have been implicated in many important pathologies, including diabetes, obesity, neurodegenerative disorders, viral infection and a variety of ER storage diseases.[5,17–21] However, the regulation and execution steps of ER stress-induced apoptosis in mammals remain poorly understood, making this area an important topic for future research.

The ESR in Yeast

The foundation of our knowledge of the ESR rests on the genetic analysis of the budding yeast *Saccharomyces. cerevisiae*.[2,3,22] In yeast, ER stress is monitored by Ire1p, an ER transmembrane protein with an N-terminal lumenal domain and a C-terminal serine/threonine kinase and endonuclease domains in the cytoplasm or nucleus.[23,24] Normally, the ER lumenal domain of a single Ire1p molecule interacts with Kar2p, an ER lumenal chaperone of the Hsp70 family that assists in folding nascent polypeptides entering the lumen.[25] Under ER stress conditions, such as when unfolded protein accumulates inside the ER, Kar2p is titrated away from Ire1p by unfolded protein.[25] The removal of Kar2p permits the oligomerization of Ire1p through its lumenal domain and the subsequent activation of Ire1p and *trans*-autophosphorylation via the C-terminal kinase domain.[23–25]

Activated Ire1p signals to the nucleus to initiate the transcription-dependent UPR. The endonuclease domain of activated Ire1p splices the mRNA of the HAC1 gene, which encodes a bZIP transcription factor that drives expression of UPR-responsive genes.[26–29] HAC1 mRNA is constitutively transcribed under resting conditions but contains an intron with secondary structure that blocks translation.[26,28,29] During ER stress, the translation attenuator intron is spliced from the HAC1 message by activated Ire1p in a novel reaction that is independent of the spliceosome but dependent on tRNA ligase.[26,30] The splicing of HAC1 mRNA both facilitates its translation and causes a frame-shift that replaces the C-terminal portion of the protein with a more potent transactivation domain, thereby promoting efficient UPR induction by mature Hac1p in two distinct ways.[26,29,31]

DNA microarray analyses have shown that genes involved in all stages of the secretory pathway are upregulated by Hac1p, including regulators of protein folding, translocation, glycosylation, vesicular transport, cell wall biosynthesis, vacuolar protein targeting and phospholipid metabolism.[32,33] These targets of Hac1p reduce the burden on the ER by increasing the capacity and efficiency of protein folding and export in the lumen. Remarkably, an estimated 5% of all yeast genes are induced in response to ER stress,[32] suggesting that the accumulation of unfolded proteins in the ER may remodel global cell physiology.

In addition to upregulating components of the secretory pathway, ER stress also activates a novel proteolytic pathway known as ERAD.[3,8–11] In ERAD, misfolded proteins in the ER membrane or lumen are actively retro-translocated, or 'dislocated', into the cytoplasm, where they are targeted for ubiquitin-mediated degradation by the proteasome. ERAD can be divided into four conceptual steps: the recognition and targeting of an unfolded substrate to the dislocation apparatus, its transport across the ER membrane, its release into the cytosol and its degradation.[8] The components that mediate each step are not fully understood and, in fact, appear to vary by substrate, including differences between soluble lumenal and membrane proteins.[34] However, recent research has provided important insight into the major features of the ERAD machinery.

The recognition and targeting of irreparably misfolded polypeptides to the dislocation machinery are mediated, at least in part, by protein glycosylation and chaperone proteins in the ER lumen. In particular, chaperones such as Kar2p and protein disulfide isomerase 1 (PDI1) play an important role in the case of soluble ERAD substrates and perhaps integral membrane proteins as well.[35–38] In some cases, glycosylation of misfolded proteins is also necessary for recognition by the ERAD machinery, possibly via a distinct set of chaperones.[39,40] However, the precise reason for this requirement is not yet clear.

Some experiments indicate that the dislocation step itself occurs, at least in some instances, through the Sec61 channel, which also mediates the entry of nascent polypeptides into the lumen from ER-associated ribosomes.[41,42] Both genetic and biochemical evidence suggests that several ERAD substrates require Sec61 function, implying that the channel may act bidirectionally.[35,43,44] However, the impairment of ERAD in some Sec61 loss-of-function genetic systems may be an indirect effect of the disruption of ER homeostasis due to blocked import of ER-targeted proteins, rather than to a direct effect on an ERAD dislocation channel. In addition, it is unclear whether all ERAD substrates require Sec61 to exit the ER, nor is there any mechanistic model for how soluble misfolded proteins in the lumen could be threaded into the Sec61 channel.[8] Therefore, other means of ER egress may exist.

The release and ubiquitination of ERAD substrates are likely coordinated.[8–10] Cdc48p, a cytosolic member of the AAA family of ATPases, participates in pulling ERAD substrates out of the ER in an ATP-dependent manner in cooperation with Ufd1p and Npl4p.[45–49] In concert with Cdc48p action, ERAD substrates are ubiquitinated and targeted for degradation via the proteasome. Ubiquitin conjugating enzymes participating in ERAD include Ubc1p, Ubc6p, Ubc7p and its membrane-anchored binding partner Cue1p.[50–52] Other proteins physically and genetically interact with the ubiquitin-conjugating machinery during dislocation, including Der1p, Der3p/Hrd1p and Hrd3p,[53–56] although the exact biochemical functions of all these components in yeast are unknown. In addition to signaling for degradation, ubiquitination may also assist in removing some substrates from the ER via a ratchet mechanism, whereby polyubiquitinated chains are prevented from passively sliding backwards through the dislocation channel. Consistent with this model, inhibiting polyubiquitination causes the accumulation of ERAD substrates inside the ER.[46,50]

ERAD and the UPR are reciprocally regulated. On the one hand, DNA microarray experiments showed that critical ERAD genes, such as DER1 and HRD3, are strongly induced by treatment with the N-linked glycosylation inhibitor tunicamycin (Tm) in wild-type but not *ire1*Δ yeast, indicating that the UPR can upregulate ERAD.[32,52] Indeed, the *ire1*Δ defect was overcome by forced expression of mature Hac1p, implicating the core signaling machinery of the UPR in ERAD induction.[32,33] On the other hand, in ERAD-defective strains, such as *ubc1*Δ, *ubc7*Δ, *der1*Δ, *hrd1*Δ or *hrd3*Δ, the UPR is constitutively activated, indicating a feedback control of ERAD over the UPR.[32,52] Therefore, in ERAD-deficient mutants, misfolded protein in the ER cannot be removed, causing the cells to activate the UPR to restore ER homeostasis. Importantly, single mutations impairing either the UPR or ERAD do not grossly affect yeast cell viability, but combining them results in synthetic toxicity, in part through the accumulation of reactive oxygen species (ROS) in the ER.[32,52,57] Thus, ERAD and the UPR cooperate and the ESR is critical for cell viability, even in the absence of unusual, exogenous stress.

For years, it was thought that yeast lacked the third major ESR pathway outlined above, that of translational control. Recently, however, a bioinformatic approach revealed that the transcription factor Gcn4p is upregulated by ER stress via a translational mechanism and is required for the full activation of a variety of UPR genes.[58] The best known role of Gcn4p is in the response to amino-acid starvation, which causes the translational upregulation of GCN4 mRNA through the activity of Gcn2p, a kinase that phosphorylates the eukaryotic translation initiation factor 2 subunit α (eIF2α).[59] Upon its induction, Gcn4p upregulates the expression of amino-acid

biosynthesis and transport genes.[59] Interestingly, it was recently shown that the same Gcn2p/eIF2α/Gcn4p pathway is also activated by ER stress and participates in UPR induction, demonstrating that a form of translational control exists in the yeast ESR.[58] Further work is needed to determine whether the phosphorylation of eIF2α in yeast has important consequences during ER stress beyond Gcn4p upregulation, as it does in mammals.

ESR in Mammals

The mammalian ESR retains several key features of the yeast program but is significantly more complex (see Figure 1).[2–6,13] Mammals have two homologs of Ire1p (Ire1α and Ire1β) and a homolog of Kar2p (BiP/Grp78), which serve as sentinels of ER stress and are believed to function similarly to the corresponding yeast proteins. Ire1α is ubiquitously expressed, while Ire1β is detected only in the intestine.[60,61] Like yeast Ire1p, the overexpression of mammalian Ire1α activates the UPR in the absence of any ER stress signal.[60] However, while Ire1p is absolutely required in yeast for initiation of the UPR, cells derived from Ire1$\alpha^{-/-}$/Ire1$\beta^{-/-}$ embryos show no obvious UPR defect, demonstrating that compensatory pathways exist.[13]

The mammalian X-box-binding protein-1 (XBP-1), a bZIP member of the CREB/ATF family of transcription factors, serves as a functional homolog of yeast Hac1p.[62,63] XBP-1 mRNA is ubiquitous in adult tissues but preferentially expressed in the fetal exocrine glands, osteoblasts, chondroblasts and liver.[64] Like HAC1, newly synthesized XBP-1 transcript must be spliced by activated Ire1 to a mature, efficiently translated mRNA.[62,63] When activated by ER stress, Ire1 excises an intron from the immature XBP-1 transcript, resulting in the replacement of the C-terminal domain of the protein and the translational activation of the mRNA, analogous to the splicing of HAC1 mRNA.[62,63,65]

Unlike HAC1 mRNA, mammalian XBP-1 mRNA is not expressed at high levels in unstressed cells.[63] Instead, the expression of XBP-1 mRNA is regulated by the transcription factor ATF6, which is itself an important component of the ESR.[63,66–68] The overall structure of ATF6 does not resemble Hac1p and ATF6 protein levels are not regulated by mRNA processing.[67] However, ATF6 does share significant sequence identity with Hac1p in its N-terminal basic region and ATF6 overexpression activates many targets of the mammalian UPR.[66–69]

Like HAC1 and XBP-1, ATF6 is activated by ER stress via an unusual mechanism. ATF6 is a type II transmembrane

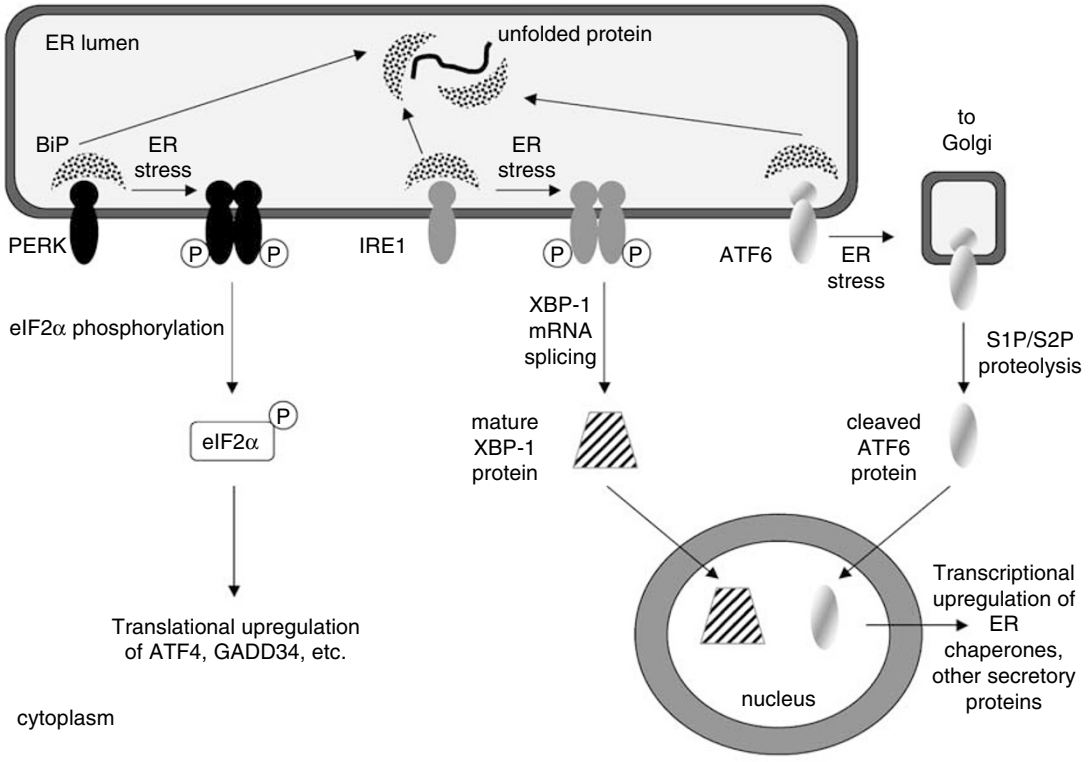

Figure 1 Mammalian ESR signaling. Unfolded protein in the ER lumen titrates BiP away from three sentinels of ER stress: PERK, IRE1 and ATF6. Activated PERK phosphorylates the translation initiation factor eIF2α to slow global protein synthesis temporarily and upregulate certain stress-inducible messages, such as ATF4. Activated IRE1 splices the mRNA for XBP-1 to allow the translation of mature XBP-1 protein, a transcription factor that mediates the transcriptional upregulation of numerous genes involved in mammalian ER function and the secretory pathway in general. Similarly, during ER stress ATF6 trafficks to the Golgi, where it is cleaved by S1P/S2P proteases and thereby released from the membrane to activate a distinct but overlapping set of genes in the nucleus. See text for details

protein in the ER of unstressed cells.[67] As with Ire1, the ER lumenal domain of ATF6 interacts with BiP, which binds to a Golgi localization signal (GLS) sequence on ATF6.[70] During ER stress, BiP is titrated away from ATF6, exposing the GLS and allowing ATF6 to traffic to the Golgi.[70,71] There, ATF6 is cleaved by the site-1 and site-2 proteases, the same enzymes that cleave membrane-bound sterol response element binding proteins during conditions of sterol starvation.[67,71] When ATF6 is cleaved, its N-terminal bZIP domain is liberated from the membrane and enters the nucleus to activate transcription of XBP-1 and other UPR target genes.[67,71] Interestingly, time course studies have shown that ATF6 action precedes that of XBP-1 during ER stress, suggesting a time-dependent shift in the UPR transcriptional response.[72] ATF6 and XBP-1 therefore play distinct but complementary roles in adjusting UPR-mediated transcription during persistent stress.

Mammalian ERAD pathways are less well understood than those of yeast, but several parallels are clear.[8,9] As in yeast, glycosylation probably participates in the recognition of mammalian ERAD substrates,[73] as do BiP and PDI.[74,75] Similarly, at least some mammalian ERAD substrates may be dislocated through the Sec61 channel.[76,77] Ubiquitination machinery similar to that of yeast is also employed, and a homolog of Cdc48p, termed p97, has been shown to cooperate with mammalian homologs of Ufd1p and Npl4p in substrate dislocation.[78–80] Recently, two groups using independent functional criteria identified Derlin1 as a mammalian homolog of Der1p and demonstrated that it mediates the association of p97 with the ER membrane to facilitate ERAD.[81,82] Another protein in this p97 targeting complex, VIMP, was also identified in one of these studies.[82]

As might be expected, mammalian ERAD pathways are more complex than those of yeast and several novel components have been described. For example, several groups recently identified the U-box protein CHIP as a ubiquitin E3 ligase that binds to chaperone and E2 enzymes to mediate ERAD.[83–86] Interestingly, Parkin, a gene associated with a recessive form of juvenile Parkinson's disease (PD), encodes another ERAD ubiquitin E3 ligase.[87–89] Parkin mediates the ERAD degradation of a novel form of α-synuclein, a protein also implicated in inherited forms of PD and the major constituent of Lewy inclusion bodies observed in the neurons of PD patients.[87] Parkin mutants associated with familial PD are unable to act on α-synuclein, suggesting that Parkin-mediated ERAD processing of α-synuclein is critical for avoiding PD.[87] It is likely that other substrate- and cell type-specific components of the mammalian ERAD pathway remain to be identified.

The third major pathway of the mammalian ESR depends on the control of protein translation.[13] As in yeast, translational control in the mammalian ESR is mediated by the transient phosphorylation of the translation initiation factor eIF2α. EIF2 is a tripartite protein complex that binds and hydrolyzes GTP during its role in recruiting the initiator methionyl-tRNA to the 40S ribosome to begin mRNA translation.[90] In order to initiate subsequent rounds of translation, eIF2 must exchange its bound GDP for GTP, a process facilitated by the guanine nucleotide exchange factor (GEF), eIF2B.[90–92] However, when the α subunit of eIF2 is phosphorylated on Ser51, it binds to eIF2B with much greater affinity.[90–92] This interaction physically sequesters eIF2B, which is stoichiometrically limiting in the cell, and prevents it from mediating the exchange of GDP for GTP by eIF2. Therefore, phosphorylated eIF2α downregulates global translation by inhibiting its own GEF.

Mammals have four eIF2α kinases that respond to diverse stress stimuli: GCN2, PKR, HRI and PERK. Like its counterpart in yeast, mammalian GCN2 is activated during amino-acid starvation by binding to uncharged tRNAs.[93,94] PKR is activated by double-stranded RNAs and is an important component of the interferon-mediated antiviral response.[95,96] HRI is restricted to erythroid cells and is inhibited by heme, such that it blocks the translation of new globin chains when insufficient heme exists to assemble holo-hemoglobin.[97] Finally, PERK phosphorylates eIF2α in response to ER stress.[98]

Like Ire1, PERK is a type I ER transmembrane protein.[99] The large ER lumenal domain of PERK is homologous to that of Ire1α and Ireβ, implying that the PERK activation mechanism may resemble that of mammalian and yeast Ire1 proteins.[25,100] Indeed, the lumenal domains of Ire1 and PERK interact with BiP under resting conditions but dimerize in a ligand-independent manner during ER stress.[25,100,101] As in yeast, it is believed that excess unfolded protein accumulates during ER stress and competes with mammalian Ire1 and PERK for BiP binding, allowing Ire1 or PERK to homodimerize and self-activate by *trans*-autophosphorylation. Consistent with this model, the displacement of BiP from Ire1 or PERK correlates with the appearance of activated PERK and Ire1, and overexpression of BiP attenuates their activation.[25]

The transient phosphorylation of mammalian eIF2α (e.g., by PERK) downregulates the translation of most mRNAs. It has been suggested that this relatively brief window of translation attenuation alleviates the burden on the stressed ER by reducing the traffic of newly synthesized polypeptides into the organelle.[99] Indeed, PERK$^{-/-}$ cells are hypersensitive to ER stress.[102] Paradoxically, however, some components of the ESR are induced at the translational level when eIF2α is phosphorylated, including the transcription factor ATF4 and its downstream target CHOP, comprising a translation-activated response that promotes cell survival during ER and other stresses.[93,103]

Although ATF4 mRNA is constitutively expressed, it is normally associated with monoribosomes and low molecular weight polyribosomes, indicating that it is inefficiently translated under resting conditions.[93,103] However, ATF4 mRNA quickly shifts to heavier ribosomal fractions and is selectively translated in a PERK-dependent fashion upon ER stress induction.[93] Ribosomal bypass scanning of the upstream open reading frames (uORFs) in the 5′ leader is thought to allow the preferential translation of ATF4 mRNA even when most protein translation is halted by eIF2α phosphorylation.[93] Indeed, this mechanism is reminiscent of the upregulation of GCN4 mRNA in yeast by Gcn2p-mediated eIF2α phosphorylation.[58,59,90] Therefore, while eIF2α phosphorylation inhibits the efficient translation of most mRNAs, it activates the translation of a small subpopulation of transcripts that contain small uORFs, such as ATF4.[59,90,93,104,105] Accumulating evidence suggests that this gene expression program is the major cytoprotective effect of eIF2α phosphorylation.

Genetic experiments indicate that ATF4 is not the only important gene subject to translational upregulation by the PERK/eIF2α phosphorylation pathway. Indeed, overexpression of ATF4 is insufficient to activate the full complement of ESR genes[93] and the gross phenotype of ATF4$^{-/-}$ mice[106] is different from that of PERK$^{-/-}$ mice[99] and eIF2α$^{A/A}$ knockin mice, which harbor a homozygous Ser^{51}→Ala^{51} mutation at the critical phosphorylation site in the endogenous eIF2α locus.[107] EIF2α$^{A/A}$ cells fail to upregulate about one-third of the genes normally induced by ER stress and are hypersensitive to ER stress-induced apoptosis,[107] indicating that translational control impacts on a wide range of ER stress-responsive genes. However, eIF2α$^{A/A}$ cells also show transcription profile differences from wild-type cells under unstressed conditions,[107] so the immediate consequences of the loss of eIF2α phosphorylation during ER stress remain to be dissected in detail. On the other hand, ATF4 is critical for the full response to stresses that induce eIF2α phosphorylation,[108] probably through the direct transcriptional upregulation of UPR targets such as BiP and CHOP.[108–110]

CHOP is a bZIP transcription factor that contains an ESR element in its promoter and is transcriptionally upregulated by ATF6.[109,111,112] Interestingly, CHOP protein induction also stringently depends on eIF2α phosphorylation, probably both because CHOP transcription is also activated by ATF4,[109] and because the 5′ leader of the CHOP mRNA contains small uORFs.[93,113] CHOP can form heterodimers with members of the C/EBP and fos-jun families of transcription factors[114,115] and likely contributes to the regulation of many genes in orchestrating the transcriptional component of the ESR.[116]

EIF2α signaling is also controlled by protein dephosphorylation mediated by the general cellular serine/threonine phosphatase protein phosphatase 1 (PP1).[117,118] The catalytic subunit of PP1 shows little, if any, intrinsic substrate specificity and relies on the binding of non-enzymatic cofactors to direct its subcellular localization and substrate choice.[119] In the case of eIF2α dephosphorylation, GADD34 functions as a cofactor for PP1 under ER stress conditions.[118] Like CHOP and ATF4, the expression of GADD34 itself is activated by eIF2α phosphorylation, and GADD34 induction requires GCN2 or PERK under conditions of amino-acid starvation or ER stress, respectively.[118] At least in some contexts, this may be mediated by upregulation of GADD34 mRNA by CHOP[116] and/or the transcription factor ATF3, which is also upregulated by ATF4.[120] Therefore, the regulation of eIF2α by GADD34/PP1 completes a feedback loop to control translation: during ER stress, PERK phosphorylates eIF2α, which induces the expression of ATF4, ATF3 and CHOP. These transcription factors then upregulate GADD34, which mediates the dephosphorylation of eIF2α by PP1. Thus, eIF2α phosphorylation is normally transient and protein translation returns to its baseline state after ER stress has abated. Consistent with this model, GADD34$^{-/-}$ cells are impaired in their ability to resume normal translation following ER stress.[116,121,122]

Other proteins can mediate eIF2α dephosphorylation as well. A constitutively expressed homolog of GADD34, termed CReP, binds to PP1 and keeps eIF2α unphosphorylated in the absence of stress.[123] Indeed, RNAi against CReP induces eIF2α phosphorylation in unstressed cells.[123] More recently, the SH2/SH3 domain-containing protein Nck-1 has been shown to antagonize PERK signaling in a phosphatase-dependent manner, probably through the direct dephosphorylation of activated PERK itself.[124] Nck-1 activity also results in the dephosphorylation of eIF2α though whether it does so indirectly, by inactivating PERK, or directly, by mediating eIF2α dephosphorylation (or both), remains to be determined.[124]

The bZIP transcription factor Nrf2 was recently identified as a second substrate of PERK.[125] Upon ESR induction, PERK phosphorylation causes Nrf2 to relocalize from the cytoplasm to the nucleus, where it upregulates a range of antioxidant response genes.[126–128] Therefore, a loss of Nrf2 function may partly explain why PERK$^{-/-}$ cells experience increased oxidative stress in response to perturbations in ER function.[108] Consistent with this model, Nrf2$^{-/-}$ cells are sensitized to ER stress,[125] providing another way in which PERK signaling promotes cell survival.

ESR and Apoptosis

The ESR acts to restore normal ER homeostasis and is therefore cytoprotective. However, when a stress is so strong or persistent that ER dysfunction cannot be corrected, metazoan cells can initiate apoptosis, allowing the regulated destruction of cells that are irreparably damaged or a risk to the organism as a whole. A unified model for ER stress-induced apoptosis is only beginning to emerge, but recent interest in the field has generated an increasing amount of information (see Figure 2).

Some core components of the protective ESR participate in ER stress-induced apoptosis as well. For example, mammalian Ire1 can activate JNK and downstream proapoptotic kinases such as ASK1, which may contribute to ER stress-induced apoptosis, as it does in response to other stimuli.[129,130] ER stress inducers such as Tm, thapsigargin (Tg, an inhibitor of the ER calcium-dependent ATPase) or reducing agents, as well as the overexpression of Ire1α, induce the activation of JNK.[130] Ire1 also interacts with TRAF2, an adaptor protein involved in the signaling pathways of proinflammatory cytokines.[130] This interaction recruits ASK1 to form an Ire1/TRAF2/ASK1 ternary complex, which in turn could activate JNK.[130,131] The functional importance of JNK activation in the ER stress pathway has not been fully explored, but ASK1$^{-/-}$ cells are partially resistant to ER stress-induced apoptosis,[132] suggesting that JNK may promote apoptosis in this context. A recent report also indicates that ATF6 might mediate a proapoptotic signal during normal myoblast development,[133] implicating this upstream UPR sentinel in apoptotic pathways as well.

CHOP has also been shown to promote apoptosis in several contexts,[93,112,134] and this effect can be blocked by BiP overexpression,[111] indicating that CHOP-activated apoptotic pathways are downstream from the ER. CHOP can transcriptionally downregulate the antiapoptotic protein Bcl-2[135] and upregulate DR5, a member of the death receptor protein family.[136,137] Interestingly, CHOP also leads to a depletion of cellular glutathione and an increase in ROS in

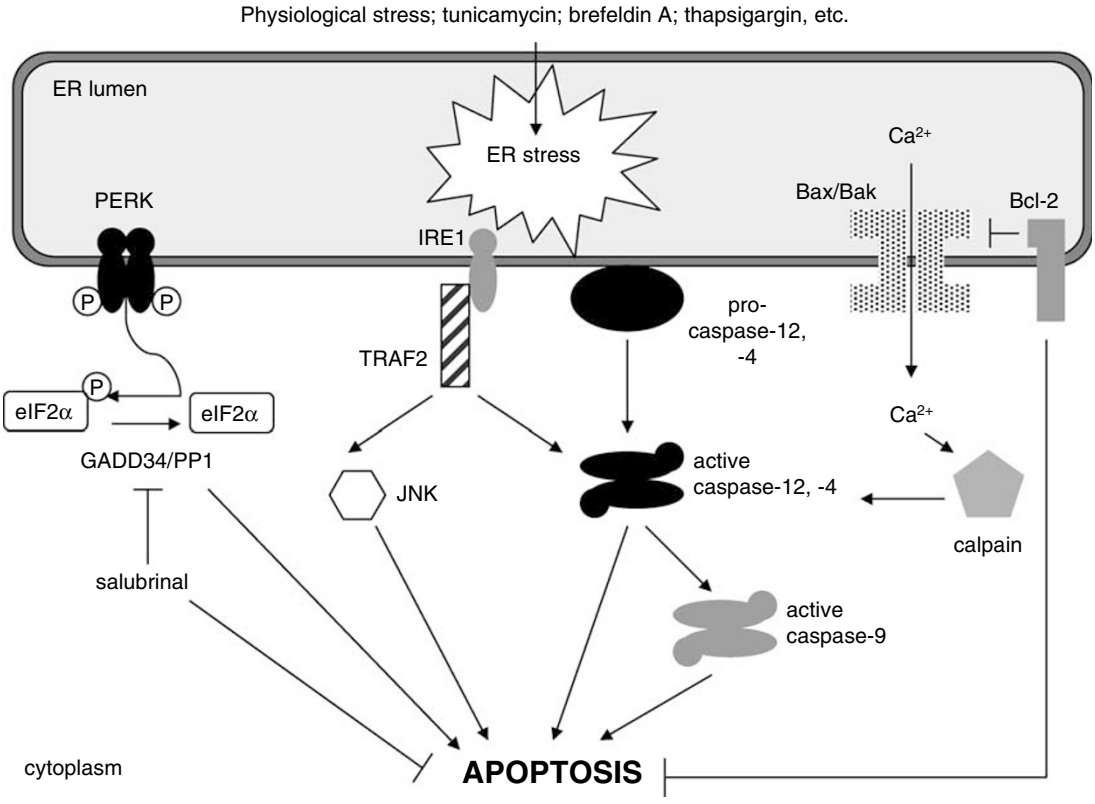

Figure 2 Simplified depiction of selected apoptotic pathways induced by ER stress. Physiological or experimentally induced ER stress leads to the activation of PERK and, eventually, the GADD34/PP1 phosphatase complex, which dephosphorylates eIF2α, promoting apoptosis. Genetic strategies or chemicals (e.g., salubrinal) that enforce eIF2α phosphorylation protect cells from ER stress-induced apoptosis. Caspase-12 (mice) or -4 (humans) is associated with the cytoplasmic face of the ER membrane and can be activated by ER stress in several ways, including via IRE1 and TRAF2, or by cleavage by calpain, itself activated by the release of calcium from ER stores. Bcl-2 family members also reside in the ER membrane and influence apoptosis induced by ER stress, both through the regulation of calcium flux and amplification of the apoptotic signal via the mitochondrial pathway (not shown). See text for details

the ER, due in part to its induction of ERO1α, an ER oxidase.[116,135] Interfering with ERO1α function reduces the accumulation of ROS in the stressed ER, leading to cytoprotection.[108] Therefore, ERO1α may be an important apoptotic effector downstream of CHOP. $CHOP^{-/-}$ MEFs are partially resistant to ER stress-induced cell death, although $CHOP^{-/-}$ mice are not resistant to lethal doses of Tm,[112] suggesting that other proapoptotic pathways are also at work.

The eIF2α phosphatase cofactors GADD34 and CReP also mediate apoptotic signaling downstream from the ER. During ER stress, $GADD34^{-/-}$ cells display persistent eIF2α phosphorylation[121,122] and fewer misfolded protein aggregates in the ER lumen,[116] suggesting that GADD34 function and the removal of eIF2α phosphorylation are proapoptotic in this context. Indeed, $GADD34^{-/-}$ mice are resistant to the toxic effects of Tm.[116] Similarly, RNAi-mediated knockdown of CReP protects cells from a variety of stimuli, including ER stress.[123] It seems likely that the cytoprotection provided by genetic loss of GADD34 or CReP is due primarily to increased eIF2α phosphorylation because enforcing eIF2α phosphorylation with constitutively active PERK had a similar effect.[138] Consistent with this hypothesis, we have shown that a selective pharmacological inhibitor of eIF2α phosphatases, termed salubrinal, can protect cells from ER stress-induced apoptosis.[139]

ER stress can also activate well-known general regulators of mammalian apoptosis, including the Bcl-2 and caspase families of proteins. It has long been known that a pool of endogenous Bcl-2 resides in the ER membrane,[140,141] and while Bcl-2 family members are thought to function principally at the mitochondrial outer membrane,[142] there is ample evidence that they influence homeostasis and apoptosis from the ER as well.[143,144] For example, variants of the antiapoptotic family members Bcl-2 or Bcl-X_L targeted specifically to the ER membrane can block apoptosis induced by pharmacological kinase inhibition or by proapoptotic Bcl-2 family members.[145,146] Conversely, ER stress itself can upregulate or otherwise activate several 'BH3-only' proapoptotic members of the Bcl-2 family, including Bim,[147] BIK,[148–151] and PUMA.[152,153] Therefore, efferent signaling from the stressed ER can engage the Bcl-2 death machinery directly.

Several studies have also demonstrated that the proapoptotic multidomain Bcl-2 family proteins, Bax and Bak, regulate ER stress-induced apoptosis. $Bax^{-/-}/Bak^{-/-}$ MEFs are remarkably resistant to many forms of apoptosis, including ER stress,[154,155] implying that many apoptotic signaling pathways converge on Bax and Bak. Interestingly, endogenous Bax and Bak regulate ER stress-induced apoptosis from both the mitochondrial and ER membranes. In the ER membrane, Bax and Bak are crucial for maintaining the

resting level of lumenal calcium, probably through an interaction with the type 1 inositol trisphosphate receptor.[156–160] As a result, $Bax^{-/-}/Bak^{-/-}$ cells display reduced calcium release from the ER in response to such stimuli as arachidonic acid and oxidative stress, thereby attenuating apoptosis.[159] However, in response to other ER stress stimuli, such as the ER-to-Golgi vesicle transport inhibitor brefeldin A (BFA), Bax and Bak must be present at both the ER and mitochondrial membranes for apoptotic execution to proceed normally.[159] Therefore, Bax and Bak participate in signal integration between the ER and the mitochondria to influence cell survival choices from multiple locations within the cell. Interestingly, a recent study also suggested that Bak but not Bax can mediate structural changes in the ER itself in a Bcl-X_L-dependent manner, suggesting a unique role for Bak in regulating ER homeostasis.[161]

The caspase family of proapoptotic cysteine proteases also plays a critical role in ER stress-induced apoptosis. Caspase-12, a murine protein associated with the ER membrane, is activated by ER stress-induced apoptosis but not by other non-ER stimuli[162] and is required for cell death in response to both pharmacological ER stress[162] and ER-targeted Bim.[147] However, caspase-12 can be activated by ER stress in several ways. For example, the cytoplasmic calcium-activated protease calpain can cleave and activate caspase-12 in response to calcium flux from the ER, which is often triggered by ER stress.[163] Interestingly, caspase-12 may also autoactivate through a direct association with Ire1α and the adaptor protein TRAF2,[164] although how ER stress regulates the formation of this complex is not yet clear. Caspase-12 has also been detected in high molecular weight complex with apoptosis-linked gene-2 protein and p97 (also referred to as valosin-containing protein), the ERAD mediator.[165] Interference with the formation of this complex protects cells from ER stress-induced apoptosis, presumably by blocking the activation of caspase-12 or caspase-9.[165] Because p97 is also involved in ERAD, it may also mediate crosstalk between the prosurvival and proapoptotic pathways induced by ER stress.

Once activated, caspase-12 can initiate downstream apoptotic pathways. For example, ER stress can induce the activation of caspase-9 independent of Apaf-1, the usual mediator of caspase-9 activation.[166] This probably occurs via the direct cleavage of caspase-9 by caspase-12.[167] In addition, caspase-7 translocates to the ER in response to some apoptotic stimuli,[168] and it has been proposed that caspase-7 can activate caspase-12 directly.[169] However, other experiments suggest that caspase-12 cleavage precedes caspase-7 cleavage during ER stress (our unpublished observations), implying that the order of activation may be the opposite in some contexts. In addition, glycogen synthase kinase 3β may influence caspase-3 activation specifically during ER stress,[170] although whether this is a direct effect or a far-upstream event remains unclear. A systematic search for caspase-12 substrates should provide additional insight into how ER stress ultimately leads to caspase-dependent cell death.

It is worth noting that the role of human homologs of caspase-12 in ER stress-induced apoptosis has been controversial.[171,172] However, it was recently shown that human caspase-4, which is 48% homologous to murine caspase-12, is localized to the ER membrane and is specifically activated by and required for ER stress-induced apoptosis.[173] These data suggest that caspase-4 is the human functional counterpart of murine caspase-12.

As noted above, the apoptotic program initiated by ER stress has been implicated in a wide variety of human diseases.[5,17–21] Other reviews in this issue of *Cell Death and Differentiation* cover the role of ER stress and apoptosis in development, metabolic disorders, neurodegeneration and viral infection in more detail.

Future Challenges

Our understanding of the mammalian ESR and the apoptotic program coupled to it has increased greatly in recent years but there remains much to learn. In addition to continuing to identify and characterize the molecular components of the ESR, investigators face several major challenges. First, we know little or nothing about how the numerous signals from the stressed ER are integrated such that the cell can 'decide' whether to initiate some or all ESR pathways, activate apoptosis or pursue a different fate altogether. In particular, it is unclear how an individual cell judges when a given level of ER stress is too great or persistent to be corrected by the ESR, making cell death the appropriate course of action. Second, many of the current cell-based models of ER stress rely on chemical agents, such as inhibitors of protein glycosylation and folding, vesicle trafficking or ER calcium flux. In contrast to these acute, synchronous, severe forms of ER stress, the physiological intensity, duration and periodicity of ER stress experienced by cells *in vivo* are quite different. Therefore, new, physiologically faithful cell-based and biochemical systems are needed to understand ESR signaling in its native context. Third, ER stress has been implicated in myriad human diseases, but the functional role of the ESR in these contexts remains largely unexplored, including questions of which ESR pathways slow disease progression, and which exacerbate it. Finally, because of the importance of the ESR to both cell biology and human health, pharmacologically useful reagents and strategies for the rational manipulation of the ESR are needed. In the future, such research tools and drug candidates may prove invaluable to basic researchers and clinicians alike.

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