



# ASIP COTRAN EARLY CAREER INVESTIGATOR AWARD LECTURE

## Multiple Mechanisms of Unfolded Protein Response—Induced Cell Death



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Eukaryotic cells fold and assemble membrane and secreted proteins in the endoplasmic reticulum (ER), before delivery to other cellular compartments or the extracellular environment. Correctly folded proteins are released from the ER, and poorly folded proteins are retained until they achieve stable conformations; irreparably misfolded proteins are targeted for degradation. Diverse pathological insults, such as amino acid mutations, hypoxia, or infection, can overwhelm ER protein quality control, leading to misfolded protein buildup, causing ER stress. To cope with ER stress, eukaryotic cells activate the unfolded protein response (UPR) by increasing levels of ER protein-folding enzymes and chaperones, enhancing the degradation of misfolded proteins, and reducing protein translation. In mammalian cells, three ER transmembrane proteins, inositol-requiring enzyme-1 (IRE1; official name ERN1), PKR-like ER kinase (PERK; official name EIF2AK3), and activating transcription factor-6, control the UPR. The UPR signaling triggers a set of prodeath programs when the cells fail to successfully adapt to ER stress or restore homeostasis. ER stress and UPR signaling are implicated in the pathogenesis of diverse diseases, including neurodegeneration, cancer, diabetes, and inflammation. This review discusses the current understanding in both adaptive and apoptotic responses as well as the molecular mechanisms instigating apoptosis via IRE1 and PERK signaling. We also examine how IRE1 and PERK signaling may be differentially used during neurodegeneration arising in retinitis pigmentosa and prion infection. (*Am J Pathol* 2015, 185: 1800–1808; <http://dx.doi.org/10.1016/j.ajpath.2015.03.009>)

The endoplasmic reticulum (ER) is an essential organelle responsible for folding of secreted and membrane proteins and lipid and sterol biosynthesis, and it is a major site of free calcium storage within the cell. Cells have evolved a unique homeostatic mechanism, termed the unfolded protein response (UPR), to ensure that the ER can adapt to changing environmental and physiological demands of its functions. In mammalian cells, the UPR is controlled by the ER resident transmembrane proteins, inositol-requiring enzyme-1 (IRE1; official name ERN1), PKR-like ER kinase (PERK; official name EIF2AK3), and activating transcription factor-6 (ATF6).

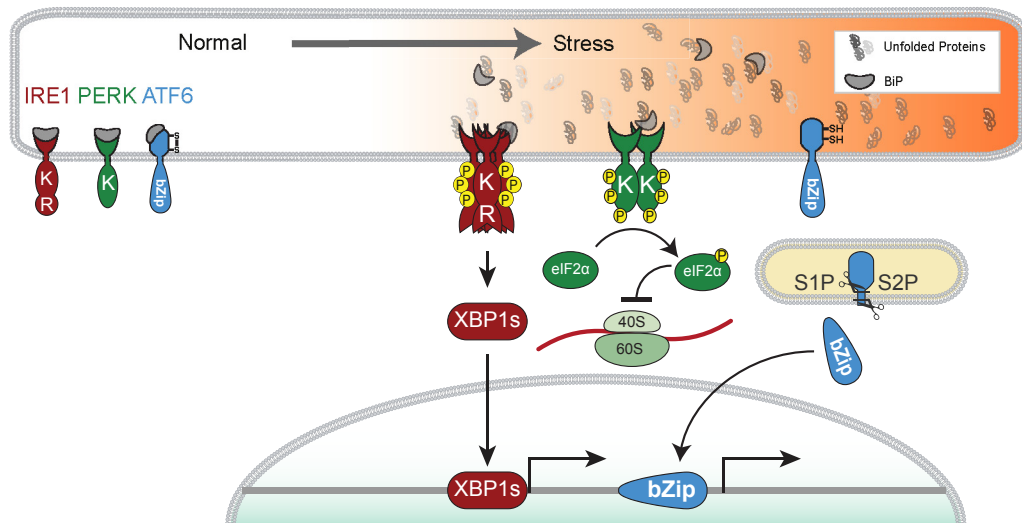
IRE1 is a transmembrane protein that controls a UPR signal transduction pathway conserved from yeast to mammals (Figure 1).<sup>1</sup> IRE1 bears a luminal domain coupled across the ER membrane to cytosolic kinase and endoribonuclease (RNase)

domains.<sup>1</sup> In response to ER stress, IRE1 undergoes oligomeric assembly, transautophosphorylation by its kinase domain, and activation of its distal RNase activity.<sup>2,3</sup> In metazoans, the RNase activity of activated IRE1 and the RtcB tRNA ligase splice out a small intron from the X-box binding protein-1 (Xbp1) mRNA to produce XBP1s transcription factor.<sup>4–8</sup>

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**Figure 1** Endoplasmic reticulum (ER) stress activates inositol-requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor-6 (ATF6) intracellular signal transduction pathways of the unfolded protein response (UPR). In normal condition, the UPR transducers, IRE1, PERK, and ATF6, associate with BiP to prevent UPR. On accumulation of misfolded proteins in the ER lumen, BiP dissociates to activate UPR transducers. IRE1 bears a luminal domain coupled across the ER membrane to cytosolic kinase (K) and endoribonuclease (RNase) domains (R). In response to ER stress, IRE1 undergoes oligomeric assembly, transautophosphorylation by its kinase domain, activating its distal RNase activity. Activated IRE1 splices out small intron from the X-box binding protein-1 (Xbp1) mRNA to generate active transcription factor XBP1s. The PERK protein bears luminal domain coupled across the ER membrane to K. In response to ER stress, PERK dimerizes and subsequently activates its cytosolic kinase domain. PERK's kinase recognizes and phosphorylates eukaryotic translation initiation factor 2 subunit alpha (eIF2 $\alpha$ ), leading to attenuation of global protein translation. ATF6 bears an ER-tethered transcription factor. In response to ER stress, ATF6 migrates from the ER to the Golgi apparatus, where site 1 and 2 proteases (S1P and S2P, respectively) cleave its luminal and transmembrane domains, and release the cytosolic portion of ATF6 containing the bZIP transcriptional activator domain. Cleaved ATF6 fragment translocates to the nucleus to serve as a transcription factor.

The transcriptional targets of XBP1 are highly enriched for ER-associated protein degradation (ERAD) factors, ER chaperones, and enzymes required for lipid biosynthesis and protein glycosylation across diverse mammalian cell types.<sup>9–14</sup> Up-regulation of these molecules by IRE1-to-XBP1s induction therefore enhances the ER's capacity to better fold new proteins as well as target irreparably damaged proteins for retrotranslocation out of the ER for degradation by proteasomes in the cytosol (Figure 1).

The ER transmembrane protein PERK regulates another UPR signaling pathway in metazoans<sup>15</sup> (Figure 1). On ER stress, PERK oligomerizes and activates its cytosolic kinase domain.<sup>15</sup> PERK's kinase phosphorylates eukaryotic translation initiation factor 2 subunit alpha (eIF2 $\alpha$ ) on Ser51, inhibiting the guanine nucleotide exchange factor eIF2B, which converts inactive GDP-bound eIF2 to its active GTP form.<sup>15,16</sup> Active eIF2 complex is needed to form the GTP-tRNA<sup>Met</sup> ternary complex required for translation initiation. Therefore, eIF2 $\alpha$  phosphorylation leads to translation inhibition that helps alleviate ER stress by reducing the load of new polypeptides that require assembly and folding in the ER compartment.<sup>17</sup>

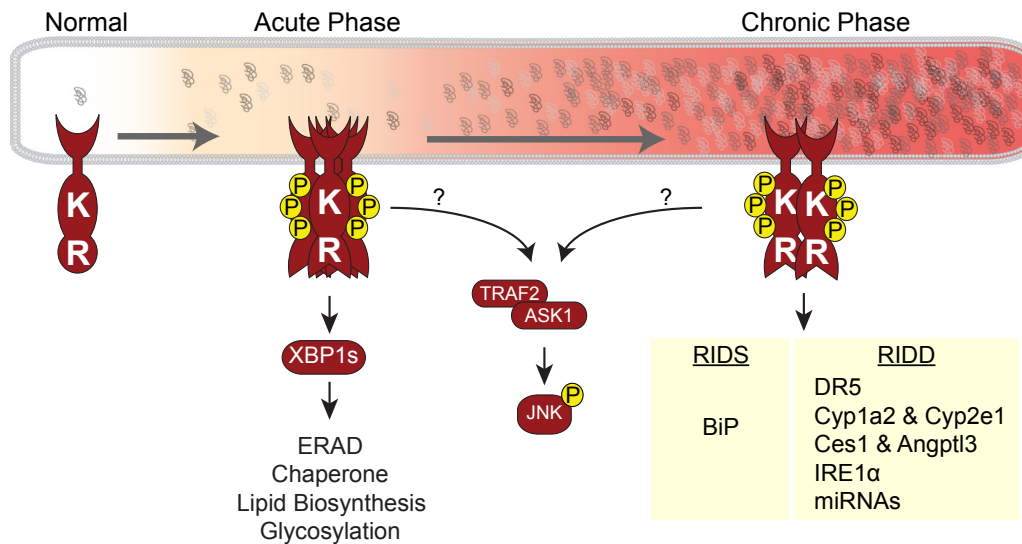
ATF6 bears an ER-tethered bZip transcription factor that regulates a third UPR signal transduction pathway<sup>18</sup> (Figure 1). In response to ER stress, ATF6 migrates from the ER to the Golgi apparatus, where site 1 protease and site 2 protease cleave its luminal and transmembrane domains to release the cytosolic portion of ATF6.<sup>18,19</sup> The cytosolic

portion of ATF6 contains the bZIP transcriptional activator domain, and after cleavage, this ATF6 fragment migrates to the nucleus to transcriptionally up-regulate ER chaperones and ERAD components, thereby enhancing ER protein-folding capacity and efficiency of ERAD.<sup>10,12,18,20</sup> Interestingly, ATF6 also transcriptionally up-regulates *Xbp1*, thereby facilitating IRE1 signal transduction by increasing levels of IRE1's RNase substrate, Xbp1 mRNA.<sup>21</sup>

Put together, these initial transcriptional and translational effects of IRE1, PERK, and ATF6 signaling help cells adapt to ER stress by enhancing the fidelity of protein folding, increasing the degradation of damaged/misfolded proteins, and suppressing new protein synthesis. However, if these actions fail to restore ER homeostasis and ER stress persists, UPR signaling consequently triggers maladaptive proapoptotic programs, many of which are specifically activated through the IRE1 and PERK pathways.

### IRE1 Signaling through RIDD, c-Jun N-Terminal Kinase, and BCL2

Seminal mechanistic studies from the laboratory of Walter and colleagues have revealed that IRE1 undergoes dynamic conformational and functional changes as a function of the duration of ER stress. In response to acute ER stress, IRE1 quickly forms oligomeric clusters in the ER plane, but IRE1



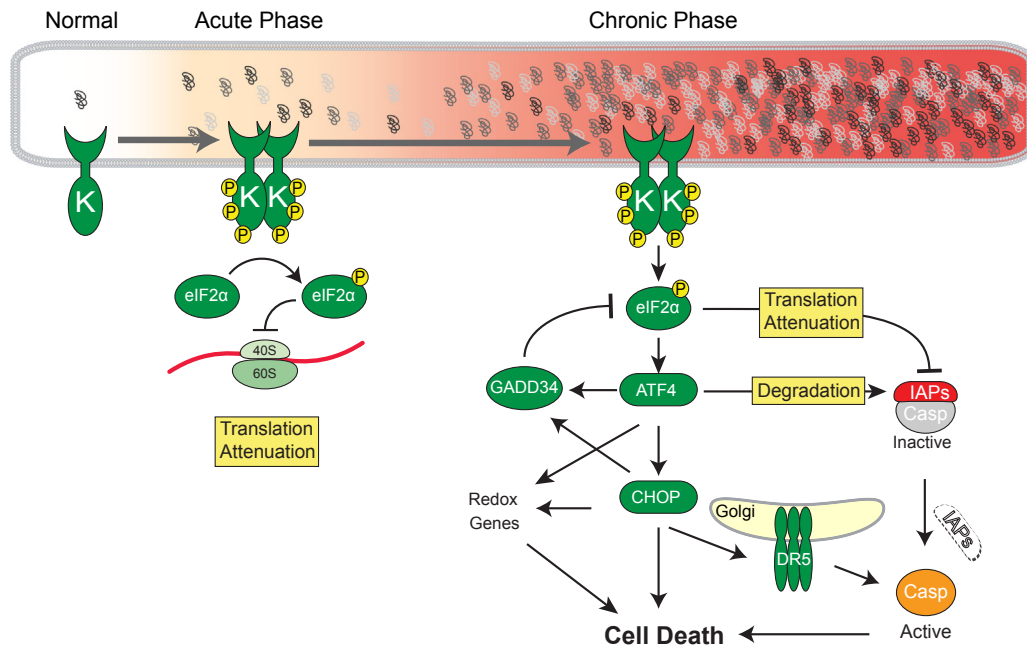
**Figure 2** Consequences of acute and chronic inositol-requiring enzyme 1 (IRE1) activation. In response to acute endoplasmic reticulum (ER) stress, IRE1 undergoes oligomeric assembly, undergoes transautophosphorylation by its kinase domain, and activates its distal RNase activity. Activated IRE1's RNase splices out a small intron from the X-box binding protein-1 (Xbp1) mRNA to produce the active transcription factor XBP1s. IRE1-to-XBP1s induction enhances the ER's capacity by up-regulation of gene sets involved in ER-associated protein degradation (ERAD), ER chaperones, lipid biosynthesis, and protein glycosylation. In the chronic phase of IRE1 activation, IRE1's RNase domain cleaves ER-targeted mRNAs in a phenomenon termed regulated IRE1-dependent mRNA decay (RIDD). Most RIDD-targeted mRNAs are disposed. In contrast, IRE1-dependent cleavage of the 3' untranslated region of BiP mRNA in *Schizosaccharomyces pombe* stabilizes BiP mRNA, thereby increasing BiP protein levels to cope with ER stress. There is likely a regulated IRE1-dependent mRNA stabilization (RIDS) rather than RIDD, which may be a new mode by which IRE1's RNase positively regulates mRNAs. The TRAF2 adaptor protein binds to IRE1 and the MAPKKK ASK1 to activate downstream molecules such as c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK). However, it is unclear which phase of IRE1 activity can interact with TRAF2-ASK1. DR5, death receptor 5.

subsequently dissociates if ER stress persists (Figure 2).<sup>22,23</sup> Interestingly, Xbp1 mRNA splicing only occurs during the acute phase.<sup>23–25</sup> During the chronic phase of ER stress, IRE1's RNase substrate specificity is altered to cleave primarily ER-targeted mRNAs in a process termed regulated IRE1-dependent mRNA decay (RIDD)<sup>26–28</sup> (Figure 2).

In contrast to Xbp1 mRNA, RIDD targets are not ligated after IRE1 cleavage, and most mRNA fragments cleaved through RIDD are degraded. RIDD's physiological significance varies widely and can confer protective or proapoptotic effects, depending on the cellular function of the mRNA being targeted. RIDD-mediated cleavage of death receptor 5 (DR5) mRNA enhances cell survival during ER stress by reducing production of proapoptotic DR5 protein.<sup>29</sup> RIDD-mediated cleavage of cytochrome P450 enzyme mRNAs in liver confers resistance to liver damage after acetaminophen overdose by preventing P450-mediated generation of hepatotoxic by-products.<sup>30</sup> The mRNA fragments produced by RIDD cleavage can trigger inflammation by engaging with the cytosolic RIG1 RNA virus innate immunity sensor.<sup>31</sup> RIDD-mediated loss of lipid metabolism mRNAs can alter plasma lipid levels in mice.<sup>32</sup> Ire1 mRNA itself is a RIDD substrate, and RIDD may act as an autoregulatory brake on IRE1 signaling by down-regulating Ire1 mRNA levels.<sup>33</sup> miRNA precursors have also been identified as RIDD substrates *in vitro*.<sup>34</sup> Disruption of miRNA maturation by RIDD cleavage may further affect multiple biological processes by modulating miRNA-mRNA interactions throughout the cell.

Recently, IRE1's RNase was found to cleave the 3' untranslated region of BiP mRNA in *Schizosaccharomyces pombe*, but this truncation surprisingly stabilized, rather than promoted, the decay of the remaining BiP mRNA, thereby increasing BiP protein levels during ER stress.<sup>35</sup> Regulated IRE1-dependent mRNA stabilization, rather than RIDD, may be a new mode by which IRE1's RNase positively regulates mRNAs (Figure 2). Recent biochemical studies have suggested that IRE1 uses RIDD at intense ER stress levels, whereas Xbp1 mRNA splicing is initiated at much lower levels of ER stress<sup>25</sup> (Figure 2). IRE1's decision to trigger RIDD, regulated IRE1-dependent mRNA stabilization, or Xbp-1 mRNA splicing may be dependent on the intensity of the stress and the ability of various cell types to respond to that stress.

The IRE1 oligomeric clusters formed by ER stress also act as molecular scaffolds to recruit other proteins and nucleate the formation of stress signal transduction sites at the ER lipid bilayer<sup>22,23</sup> (Figure 2). For example, TRAF2 adaptor protein binds to IRE1 as well as the ASK1 MAPKKK to activate cytosolic signaling kinases, such as C-Jun N-terminal kinase, p38, and extracellular signal-regulated kinase during ER stress<sup>36,37</sup> (Figure 2). IRE1 also interacts with the RACK1 adaptor protein to recruit phosphatases to the ER membrane during ER stress.<sup>38</sup> IRE1 forms protein-protein interactions with BCL2 family proteins, such as BAX and BAK, and the BI-1 BCL2 regulatory protein.<sup>39,40</sup> IRE1 also binds cytoskeletal nonmuscle myosin II during ER stress.<sup>41</sup>



**Figure 3** Consequences of acute and chronic PKR-like endoplasmic reticulum (ER) kinase (PERK) activation. PERK has a kinase domain (K), and phosphorylates eukaryotic translation initiation factor 2 subunit alpha (eIF2 $\alpha$ ). In the acute phase, PERK-eIF2 $\alpha$ P attenuates overloading the proteins into ER. On chronic activation of PERK signaling, expression of activating transcription factor-4 (ATF4) is transcriptionally up-regulated, which regulates cell fate. GADD34 dephosphorylates eIF2 $\alpha$ P to eIF2 $\alpha$ , and protein translation is reinitiated. Expression of ATF4 causes oxidative stress. Proapoptotic transcription factor CHOP is transcriptionally induced by ATF4, and its translation is also enhanced by ATF4. Death receptor 5 (DR5; official name *TNFRSF10B*) is a CHOP target gene, and abundant DR5 protein forms oligomer at the Golgi apparatus, which activates caspase (Casp)8 without requirement of any ligand. Inhibitors of apoptosis proteins (IAPs) are key cell death regulators in metazoans, through their suppression of caspases. Recent studies link PERK-eIF2 $\alpha$ P-ATF4 signaling to IAP regulation during ER stress. In response to chronic ER stress, IAP levels decrease specifically through the actions of PERK, but not IRE1 or ATF6 branches of the unfolded protein response. The eIF2 $\alpha$ P attenuates *de novo* IAP synthesis, particularly X-linked IAP, and ATF4's transcriptional activity destabilizes extant XIAP protein.

These IRE1-centered protein-protein interactions can influence the sensitivity of IRE1 activation in response to ER stress. IRE1-centered protein-protein interactions could also act conversely to influence cellular signaling processes and structures in other cellular compartments during ER stress.

### Proapoptotic Consequences of PERK Signaling

PERK signaling down-regulates translation from most mRNAs, thereby restricting *de novo* peptide loading onto the ER (Figure 1). This cytoprotective event by PERK to eIF2 $\alpha$ P occurs rapidly (Figure 3). Rare mRNAs bearing 5' upstream open reading frames, including the mRNAs encoding the ATF4, ATF5, and CHOP transcriptional activators, are paradoxically translated more efficiently during the phosphorylated state of eIF2 $\alpha$  (Figure 3).<sup>42–44</sup> ATF4's transcriptional targets include genes involved in amino acid metabolism, oxidoreductases required for disulfide bond formation in the ER, several ubiquitin ligases, GADD34 phosphatase, and CHOP transcription factor.<sup>45–47</sup> ATF4-null mouse embryonic fibroblasts are sensitive to oxidative stress and require supplemental reducing compounds for survival and growth in cell culture.<sup>46</sup> Interestingly, ATF4 overexpression in mouse embryonic

fibroblasts and neurons also evokes oxidative stress and increases cell death.<sup>45,47</sup> These findings point to ATF4 as an important determinant in regulating cell fate during ER stress, with too little and too much ATF4 both producing deleterious effects.

One mechanism by which ATF4 can promote cell death is via transcriptional up-regulation of the GADD34 phosphatase (Figure 3). GADD34 dephosphorylates eIF2 $\alpha$ P to eIF2 $\alpha$ .<sup>48</sup> Dephosphorylation of eIF2 $\alpha$ P removes the translational brake initially generated by PERK activation and leads to more protein synthesis and thereby protein folding demands on the ER. Indeed, Han et al<sup>45</sup> found that ATF4 overexpression increased protein synthesis concomitant to increasing cell death. Furthermore, chemical inhibition of GADD34's dephosphorylation of eIF2 $\alpha$ P by the salubrin and guanabenz compounds protects cells from ER stress-induced cell death.<sup>49–51</sup>

A second mechanism by which ATF4 promotes cell death is via transcription of the proapoptotic Chop gene (official name *DDIT3*), whose translation is also enhanced by eIF2 $\alpha$ P (Figure 3).<sup>16,43,44,52,53</sup> By dual transcriptional and translational up-regulation, CHOP is highly enriched when PERK is strongly activated. CHOP's role as a proapoptotic transcription factor has been clearly shown *in vitro* where CHOP-null cells are resistant to cell death induced by the chemical

ER toxins, tunicamycin, and thapsigargin.<sup>53</sup> Several of CHOP's transcriptional targets are implicated in apoptosis, including the apoptotic Bim (official name *BCL2L1*) and Puma (official name *BBC3*) Bcl-2 family genes,<sup>54,55</sup> Trb3 (official name *TAS2R13*), and Dr5 (official name *TNFRSF10B*).<sup>29,56,57</sup> DR5 can signal cell death by activating caspase-8.<sup>29,57</sup> During ER stress–induced cell death, DR5 protein accumulates in Golgi apparatus, where it oligomerizes, leading to activation of cytosolic caspase 8. Interestingly, DR5 mRNA is also down-regulated by IRE1-mediated RIDD, and the balance between the opposing effects of IRE1 and PERK on DR5 levels may tip whether UPR selects cell survival or cell death during ER stress.<sup>29</sup>

Some types of ER stress–induced damage and cell death are unlikely to be mediated via CHOP induction. Comprehensive RNA-sequencing and microarray studies saw minimal transcriptional induction of previously identified apoptotic genes after forced Chop expression.<sup>45</sup> Furthermore, forced CHOP expression itself does not trigger cell death *in vitro*,<sup>45,58</sup> indicating that other proapoptotic hits are necessary for ER stress–induced cell death.

Inhibitors of apoptosis proteins (IAPs) are key cell death regulators in metazoan organisms through their suppression of caspases.<sup>59</sup> Recent studies link PERK-eIF2 $\alpha$ -ATF4 signaling to IAP regulation during ER stress. In response to chronic ER stress, IAP levels decrease significantly in many mammalian cell types, specifically through the actions of the PERK, but not IRE1 or ATF6, branches of the UPR.<sup>60–63</sup> PERK signaling attenuates *de novo* X-linked IAP (XIAP) protein synthesis via eIF2 $\alpha$  and also promotes extant XIAP protein degradation by ATF4 transcriptional activity (Figure 3). In contrast, CHOP had no effect on XIAP levels. Loss of XIAP enhances sensitivity to ER stress–induced cell death, and overexpression of XIAP protects cells from ER stress, and interestingly, synergizes with the absence of CHOP to induce even greater resistance to ER stress–induced cell death than Chop<sup>-/-</sup> alone.<sup>58</sup> These findings show that PERK-eIF2 $\alpha$ -ATF4 signaling promotes multiple proapoptotic hits within the cell, including the induction of CHOP and suppression of IAPs (Figure 3). These effects of chronic PERK signaling, therefore, generate a cellular milieu conducive for efficient caspase activation by removal of caspase inhibitors.

The ability of IRE1 and PERK signaling to activate multiple distinct proapoptotic circuits provides attractive mechanisms to link ER stress to disease pathogenesis and progression. Physiological ER stresses vary tremendously with respect to their intensity and their cause (eg, hypoxia versus genetic mutation). Important questions for defining the role of UPR as disease mechanism include the following: Which UPR signaling events are activated by a physiological ER stress? What is the consequence of UPR activation in the cellular and tissue context of a specific disease? In the subsequent sections, we examine how UPR activation and function contribute to the pathogenesis of two

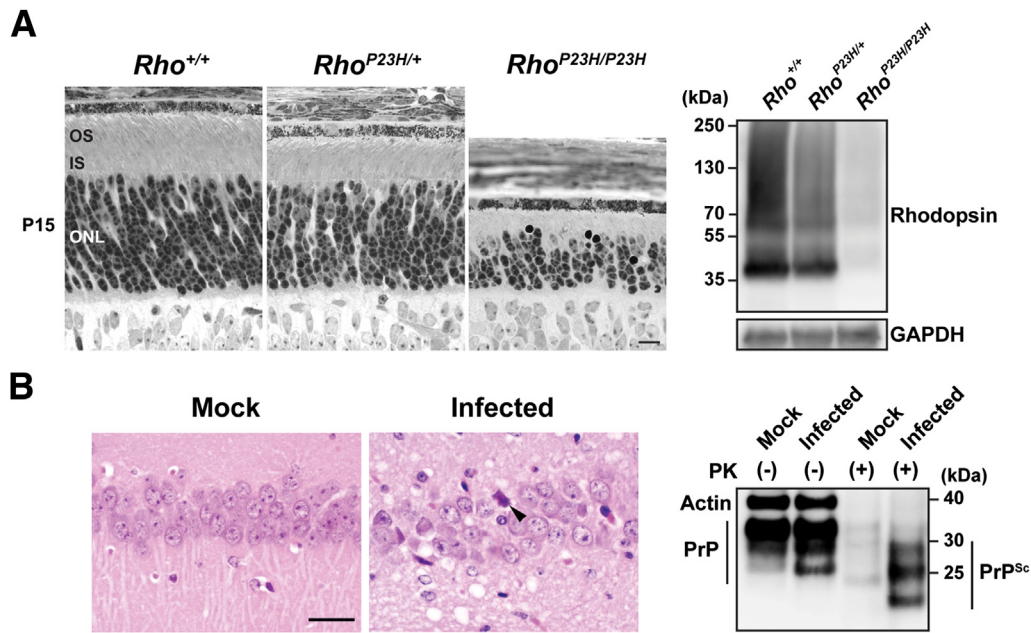
diseases associated with ER stress, retinitis pigmentosa and prion infection.

## Divergent Mechanisms of ER Stress–Induced Neurodegeneration

### ER-Associated Degradation in Retinitis Pigmentosa

Retinitis pigmentosa is a human blinding disease arising from photoreceptor cell death in the eye. Photoreceptors are specialized sensory neurons that detect light and activate retinal circuitry to transmit visual information to the brain. Photoreceptors accomplish this feat using the visual pigment, rhodopsin, a G-protein–coupled transmembrane receptor protein covalently linked to 11-*cis*-retinal.<sup>64</sup> Rhodopsin is essential for photoreceptor function and survival, and rhodopsin knockout mice (*Rho*<sup>-/-</sup>) develop early retinal degeneration.<sup>65,66</sup> More than 100 rhodopsin mutations have been identified in families with heritable types of retinitis pigmentosa.<sup>67</sup> Many of these mutations generate misfolded rhodopsin proteins that are aggregation prone and retained in the ER.<sup>24,68–71</sup> Recent studies in mouse models of retinitis pigmentosa have shed light into how the UPR in photoreceptors copes with mutant rhodopsins and influences the disease process.

The P23H rhodopsin mutation is the most common cause of heritable retinitis pigmentosa in North America, and photoreceptors carrying the mutation generate misfolded rhodopsin proteins that do not traffic normally to the rod photoreceptor outer segment. A P23H rhodopsin knock-in mouse closely mirrors the spatial distribution and temporal progression of photoreceptor cell death and vision loss found in patients with this mutation.<sup>72</sup> Analysis of these mice indicates that these photoreceptors use an unusual, customized UPR tailored to cope with P23H rhodopsin.<sup>73</sup> IRE1's induction of XBP1s, and the transcriptional up-regulation of ERAD by XBP1s, was seen in photoreceptors expressing P23H rhodopsin.<sup>73</sup> Concomitant with ERAD up-regulation, P23H rhodopsin protein is found to be robustly ubiquitinated and almost entirely degraded in these photoreceptors<sup>73</sup> (Figure 4A). In contrast, other IRE1-mediated signaling events, including c-Jun N-terminal kinase activation or RIDD, are not observed in these photoreceptors.<sup>73</sup> Furthermore, minimal activation of the PERK signaling pathway is seen, with no changes in ATF4, CHOP, or IAP levels in P23H rhodopsin-expressing photoreceptors.<sup>73</sup> These findings reveal that the dominant effect of UPR in photoreceptors of P23H rhodopsin knock-in mice is the induction of ERAD to degrade and clear mutant rhodopsin, which is accomplished through a preferential use of the parts of the UPR regulating ERAD, such as IRE1's induction of XBP1. PERK's proapoptotic functions through modulation of ATF4, CHOP, and IAPs are not used in these photoreceptors. Consistently, Chop<sup>-/-</sup> fails to delay retinal degeneration in P23H rhodopsin knock-in photoreceptors, and in other genetically modified mouse lines expressing other rhodopsin mutations seen in retinitis pigmentosa.<sup>73–75</sup>



**Figure 4** **A:** Rhodopsin (Rho) is robustly degraded during retinal degeneration. Light micrographs of wild-type and P23H knock-in mouse retinas at postnatal (P) day 15. At P15, both rod outer segments (OSs) and rod inner segments (ISs) are shorter in *Rho*<sup>P23H/+</sup> mice compared with those of the *Rho*<sup>+/+</sup> mice, and significantly shortened in *Rho*<sup>P23H/P23H</sup> mice. The outer nuclear layer (ONL) is also significantly thinner in *Rho*<sup>P23H/P23H</sup> mice. Rhodopsin protein levels are significantly diminished in *Rho*<sup>P23H/P23H</sup> mice. Retinal protein lysates were collected from *Rho*<sup>+/+</sup>, *Rho*<sup>P23H/+</sup>, and *Rho*<sup>P23H/P23H</sup> mice at P15. Rhodopsin is detected by immunoblotting. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a loading control. **B:** High prion protein (PrP) levels are maintained during prion infection. Hematoxylin and eosin-stained hippocampal sections from mock- or prion-inoculated transgenic mice expressing human PrP reveal neuronal necrosis (arrowhead) and spongiform degeneration only in prion-infected mice. Total PrP levels (PrP<sup>C</sup> + PrP<sup>Sc</sup>) in brain are similar in mock- and prion-infected mice by immunoblotting (20  $\mu$ g protein per well). Samples treated with 100  $\mu$ g/mL proteinase K (PK) reveal PK-resistant PrP<sup>Sc</sup> only in prion-infected brain (100  $\mu$ g protein per well). Actin served as a loading control (the actin bands are above the PrP in the undigested lanes). This blot was developed using monoclonal antibodies 3F4 against PrP (Millipore, Billerica, MA) and GT5412 against actin (Genetex, Irvine, CA). PrP contains two potential n-glycosylation sites and, thus, migrates as three bands corresponding to diglycosylated, monoglycosylated, or unglycosylated PrP. Scale bars: 10  $\mu$ m (**A**); 50  $\mu$ m (**B**).

What drives photoreceptor cell death if PERK's proapoptotic signals are not activated? Rhodopsin is essential for photoreceptor function, structure, development, and survival, and *Rho*<sup>-/-</sup> mice develop early retinal degeneration.<sup>65,66</sup> In P23H rhodopsin knock-in animals, rhodopsin protein degradation occurs as soon as photoreceptors are born, and loss of rhodopsin precedes any photoreceptor cell death (Figure 4A).<sup>73</sup> Disruption of rhodopsin protein homeostasis by ERAD is likely to be a key trigger for photoreceptor cell death.

### PERK Signaling in Prion Diseases

Prion diseases are fatal neurodegenerative disorders arising from conversion of the normal cellular prion protein, PrP<sup>C</sup>, into a misfolded and self-templating conformer, PrP<sup>Sc</sup>. PrP<sup>C</sup> is highly conserved among mammals and ubiquitously expressed, yet the functions attributed to PrP<sup>C</sup> are diverse and include maintenance of myelin, normal synaptic function, and neuroprotection.<sup>76–79</sup> PrP<sup>C</sup> is a glycosylphosphatidylinositol-anchored glycoprotein that undergoes folding and post-translational modifications within the ER and secretory pathway. In cell culture, PrP<sup>Sc</sup> replication triggers ER stress, resulting in the aberrant accumulation of PrP in the cytosol and further enhancing the formation of PrP<sup>Sc</sup>.<sup>80–82</sup> ER stress

and UPR activation have also been observed in prion infection *in vivo*<sup>83–85</sup> as well as transgenic mice expressing mutant PrP<sup>C</sup>.<sup>86</sup>

Genetic and chemical modulation of different UPR pathways in prion-infected models has revealed intriguing and surprising differences from mutant rhodopsin models in the role of UPR signaling pathways in cellular degeneration. PrP<sup>C</sup>-to-PrP<sup>Sc</sup> conversion and neurodegeneration are unchanged in mice deficient in neuronal *Xbp1*<sup>-/-</sup> compared with controls.<sup>85</sup> In contrast, genetic or chemical inhibition of PERK pathway signaling using GADD34 overexpression or the PERK inhibitor glycogen synthetase kinase 2606414 ameliorates neurodegeneration in prion-infected mice, whereas activating the PERK pathway using salubrinal worsens prion-associated neurotoxicity.<sup>83,84</sup> These studies reveal a direct role for the PERK pathway in prion disease pathogenesis, and suggest that IRE1 signaling, at least through XBP1s generation, is dispensable in this process.

How is the PERK signaling critical in the pathogenesis of prion disease, yet unnecessary for mutant rhodopsin-induced cell death? One fundamental difference between these diseases is that prion conversion likely occurs on the cell membrane or in an endolysosomal compartment; thus, PrP<sup>Sc</sup> escapes the misfolded protein clearance and degradation mechanisms triggered during UPR activation.<sup>86</sup>

(Figure 4B). Indeed, levels of the PrP<sup>Sc</sup> isoform increase substantially by the terminal stage of disease (Figure 4B). The accumulation of PrP in the brain likely causes chronic ER stress, leading to strong PERK signaling and its subsequent proapoptotic signaling cascade (Figure 2). In contrast, misfolded rhodopsin is degraded so quickly and efficiently (Figure 4A) that the strength and duration of ER stress do not increase to a threshold necessary for strong PERK activation. Thus, prion infection may represent a class of ER stress-associated diseases in which PERK's proapoptotic signaling output prevails when UPR protein quality control mechanisms fail to remove the misfolded proteins. Misfolded rhodopsins may represent another class of ER stress-associated diseases in which UPR protein quality control mechanisms remove the misfolded proteins but, in doing so, disrupt vital cellular structures or processes required for cell viability.

### Tailoring the UPR to Fit a Specific Disease

The UPR signaling pathways are found in all cell types and activate broad transcriptional, translational, and post-translational programs to help cells cope with ER stress. The PERK and IRE1 UPR pathways can promote cell death through multiple downstream effectors. Comparison of two ER stress-associated diseases (retinitis pigmentosa and prion disease) reveals that different UPR signaling events are activated during pathogenesis of these diseases. Customization and tailoring of the UPR to fit a physiological ER stress and specific cell types are likely to occur in other ER stress-associated disease processes.

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