IRE1: ER stress sensor and cell fate executor

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Cells operate a signaling network termed the unfolded protein response (UPR) to monitor protein-folding capacity in the endoplasmic reticulum (ER). Inositol-requiring enzyme 1 (IRE1) is an ER transmembrane sensor that activates the UPR to maintain the ER and cellular function. Although mammalian IRE1 promotes cell survival, it can initiate apoptosis via decay of antiapoptotic miRNAs. Convergent and divergent IRE1 characteristics between plants and animals underscore its significance in cellular homeostasis. This review provides an updated scenario of the IRE1 signaling model, discusses emerging IRE1 sensing mechanisms, compares IRE1 features among species, and outlines exciting future directions in UPR research.

ER stress and the UPR
The membrane trafficking system maintains the operation of approximately one-third of the eukaryotic proteome. Most secretory proteins first enter the ER for folding and assembly. To maintain the fidelity of ER functions, cells coordinate a protein quality-control system with a signaling network, the UPR [1–6]. The UPR is triggered by ER transmembrane sensors on ER stress, a cellular condition referring to the accumulation of unfolded proteins in the ER (Figure 1) [7,8]. The adaptive response occurring at the initial phase of the UPR aims to rebalance protein-folding homeostasis [2,3,9–12]. If cells fail to recover from ER stress, the UPR represses the adaptive response and triggers apoptosis [1,3,4,7,13,14]. IRE1 is the only identified ER stress sensor in yeast and is essential for the UPR in animals and plants [15–18] (Figure 1). As an ER transmembrane protein, IRE1 monitors ER homeostasis through an ER luminal stress-sensing domain and triggers the UPR through a cytoplasmic kinase domain and an RNase domain [15,16]. On ER stress, IRE1 RNase is activated through conformational change, autophosphorylation, and higher-order oligomerization [19–21]. Mammalian IRE1 initiates diverse downstream signaling of the UPR either through unconventional splicing of the transcription factor Xbp-1 or through post-transcriptional modifications via Regulated IRE1-Dependent Decay (RIDD) of multiple substrates [15,16,22–25]. In addition, PERK and ATF6 function as two distinct mammalian ER-stress sensors to cope with complex UPR scenarios [7,26] (Figure 1). Similar to IRE1, PERK, and ATF6 are ER transmembrane proteins that contain an ER luminal stress-sensing domain and a cytoplasmic enzymatic domain. To prevent a further increase in protein-folding demand in the ER, PERK transiently inhibits general protein translation through phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α). Phosphorylated eIF2α can also selectively activate translation of mRNAs including the ATF4 transcription factor to regulate UPR target genes [27]. ER stress triggers relocation of ATF6 from the ER to the Golgi, where it undergoes proteolytic cleavage. The cleaved transcription factor domain of ATF6 enters the nucleus for UPR regulation [28–30] (Figure 1).

The main molecular mechanisms underlying IRE1 unconventional splicing are conserved in eukaryotes. In budding yeast, mammals, and plants, there is only one transcription factor identified as a splicing target of IRE1 (Figure 1). The stem–loop structure and cleavage site of the IRE1-splicing substrate are conserved among species. By contrast, RIDD appears more divergent in eukaryotes. Among yeast, RIDD operates in the fission yeast Schizosaccharomyces pombe but not in the budding yeast Saccharomyces cerevisiae [31]. Intriguingly, RIDD-mediated decrease in protein-folding demand is the only identified mechanism of UPR in fission yeast [31]. Although plant RIDD may potentially degrade a significant portion of mRNAs encoding secretory proteins [32], it is undetermined whether plant RIDD processes various substrates to direct UPR outputs like mammalian RIDD. Unlike the mammalian UPR, plant PERK orthologs remain to be identified; however, two functional homologs of ATF6, bZIP28, and bZIP17, exist in plants [33] (Figure 1). Moreover, a component of the G protein complex, AGB1, is essential for the plant UPR [17] and an alternative G-protein-coupled receptor is involved in a non-canonical UPR in Caenorhabditis elegans [34]. Due to the large number of members of the mammalian G protein complex, its roles in the classical UPR might be more challenging to reveal. Although the IRE1 and ATF6 arms are partially conserved between plants and animals, it will be interesting to establish the degree of UPR diversification between the two kingdoms.

This review presents the latest advances in and viewpoints on IRE1-dependent UPR research. We focus on the recent groundbreaking discoveries that define IRE1 as a master regulator in cell fate determination under ER stress. IRE1 was long considered a positive regulator of
cell survival. Thus, repression of IRE1 was believed to potentiate apoptosis. The recent identification of novel IRE1 regulatory events reveals that IRE1 signaling is persistent during ER stress. Namely, IRE1 can no longer be considered simply as a driving force for cell survival, but rather as an administrator/executor of cell fate determination under ER stress. Through presentation of the recent evidence establishing that IRE1 triggers diverse signaling, we delineate current IRE1-signaling models. It has also become clear that IRE1 monitors cellular homeostasis beyond protein-folding status in the ER; therefore, the functional relevance of the UPR within physiological processes is discussed. Finally, we compare convergent and divergent features of IRE1 between plants and mammals to provide an integrated view of IRE1 in multicellular eukaryotes.
IRE1 signaling in cell fate determination

Life versus death determination is constantly scrutinized and tightly controlled. The prevalence of malfunctioning cells due to irremediable ER stress contributes to significant diseases, including cancer and diabetes. Conversely, overcommitment to cell death may result in organ damage or cell-degenerative diseases [35–39]. To reach optimal fitness under ER stress, cell fates are determined through tight coordination of adaptive and apoptotic responses [37,40,41]. In mammals, PERK–eIF2α–ATF4 regulates the transcription factor CHOP to activate ER stress-triggered apoptosis. In parallel, IRE1 controls cell fate determination through the mitogen protein kinase JNK under ER stress [3,7,8,42] (Figure 1). By contrast, although ER stress can play a role in programmed cell death in plants [43], very little is known about ER stress-induced cell death in plants [17,32,44]. Furthermore, lack of sequence homologs of most mammalian apoptosis regulators in plants hints that divergent mechanisms of ER stress-induced cell death exist among organisms.

Revised model of the IRE1α signaling network in mammals

The mammalian genome encodes two isoforms of IRE1, IRE1α and IRE1β. IRE1α is expressed ubiquitously and IRE1α knockout mice exhibit embryonic lethality. By contrast, IRE1β expression is restricted and IRE1β knockout mice are viable [45,46]; therefore, most mammalian UPR research is conducted on IRE1α. IRE1α was identified as a positive regulator of cell survival. It was believed that IRE1α signaling was terminated during irredeemable ER stress to enable apoptosis [1,2,7,15,47,48]. Nevertheless, recent studies have challenged this concept by showing that IRE1α persistently adjusts protein-folding capacity, actively directs UPR signaling, and executes cell fate determination [49,50] (Figure 2). IRE1α employs splicing and RIDD to direct cell fate throughout ER stress. Despite Xbp-1 being the only identified IRE1α splicing target, numerous types of RNA are proven RIDD substrates [22,49,50]. Although the significance of RIDD targets is not completely understood, some RIDD events are critical for IRE1α-dependent cell fate determination. During the adaptive response, IRE1α conducts RIDD on mRNAs encoding ER-translocating proteins to prevent further increases in protein-folding demand in the ER [50]. To augment protein-folding ability, IRE1α splices the transcription factor Xbp-1 mRNA to induce the transcription of ER quality-control components. If attempts to restore ER homeostasis fail, IRE1α ceases to splice Xbp-1 mRNA. Alternatively, IRE1α represses adaptive responses and activates apoptosis through RIDD [49,50]. During the transition phase, occurring between the adaptive and apoptotic response, RIDD increases ER stress intensity through degradation of selective UPR target genes including the ER protein chaperone BiP [50]. Once the ER stress intensity reaches its threshold, RIDD initiates apoptosis through repression of antiapoptotic pre-miRNAs [49]. Caspase-2 (CASP2) is a proapoptotic protease for the execution of apoptosis [51]. Upregulation of CASP2 is an indicator of apoptotic initiation. Through decay of anti-CASP2 pre-miRNAs, IRE1α activates apoptosis through upregulation of CASP2 (Figure 2) [49]. A close association of IRE1α activity and cell fate determination has been proposed for years [1,2,7,15,47,48]. These findings provide direct evidence that IRE1α is a molecular switch and apoptosis executioner during ER stress [49]. It was previously proposed that attenuation of IRE1 activity allows cells to initiate apoptosis [1,2,7,15,47,48]. The identification of the IRE1α–CASP2 pathway elaborates an intriguing IRE1α signaling model: IRE1α–Xbp-1 is active in the adaptive phase and attenuated in the apoptotic phase. In parallel, activation of IRE1α–CASP2 events initiates cell death in the apoptotic phase (Figure 3).

Is mammalian IRE1α the only major trigger of ER stress-induced apoptosis?

IRE1α is necessary and sufficient to trigger apoptosis, whereas PERK and ATF6 are dispensable in apoptosis activation [49]. Nonetheless, it cannot be excluded that distinct ER-stress sensors may serve as major executioners of cell death in a context-specific manner. Using chemical genetic tools, the regulatory roles of the phosphor-transfer and RNase activity of IRE1α in the UPR can be examined separately. The phosphor-transfer function is dispensable for Xbp-1 mRNA splicing and the upregulation of CASP2 expression; however, it is required for the subsequent CASP2 cleavage and apoptosis activation, indicating that the IRE1α phosphor-transfer function is essential for cell fate switching during ER stress [49,50]. Notably, the phosphor-transfer function is mostly studied through an in vitro conditional IRE1α induction that mimics ER stress. Although this experimental system is valuable to distinguish the phosphor-transfer and RNase functions of IRE1α, it is important to note that ATF6 and PERK are not activated through ER stress. A potentially compromised crosstalk among the UPR arms raises a possibility that the IRE1α induction system might not completely resemble a genuinely biological scenario of ER stress. Hence, careful data interpretation from the conditional induction system and integration of in vivo analyses are necessary to determine whether IRE1α is the master trigger in ER stress-induced apoptosis.

The substrate specificity of mammalian IRE1α

Although the four identified IRE1α-cleaved miRNAs (miR-17, miR-34a, miR-96, and miR-125b) repress the common substrate Casp2, TXNIP is another target of miR-17 [52]. TXNIP is involved in β-cell death and was selected to potentially regulate ER stress-induced apoptosis based on its rapidly elevated expression under severe ER stress. Similar to the IRE1α mutation, TXNIP mutation leads to compromised apoptosis activation, indicating that TXNIP is essential for ER stress-induced apoptosis [52,53]. Whereas PERK–eIF2α activates TXNIP transcription, IRE1α increases TXNIP expression by degradation of miR-17. Accordingly, it is conceivable that each of the four IRE1α-cleaved miRNAs might have specific substrates such as TXNIP. Based on this scenario, IRE1α might differentially degrade its individual target miRNA for fine-tuning of the UPR. Another interesting feature of mammalian RIDD is that distinct substrates have a degree of sequence similarity within the cleavage site, whereas the flanking sequences of
Figure 2. Inositol-requiring enzyme 1 alpha (IRE1α) regulatory mechanisms during endoplasmic reticulum (ER) stress. Mammalian IRE1α is repressed through a physical interaction with BiP when the demand for and capacity of protein folding is balanced in the ER. Dissociation of IRE1α from BiP due to an elevated level of unfolded protein in the ER leads to activation of IRE1α. IRE1α-activating processes include its autophosphorylation, conformational change, and higher-order assembly. IRE1α directs cell fate decisions through unconventional splicing and Regulated IRE1-Dependent Decay (RIDD). To prevent increasing demand for ER protein folding, IRE1α conducts RIDD to degrade the transcripts of ER-translocating proteins. In parallel, IRE1α unconventionally splices transcripts of the Xbp-1 transcription factor. Spliced XBP-1 enters the nucleus to transcriptionally reprogram unfolded protein response (UPR) target genes, including ER chaperones. Under irremediable ER stress, IRE1α ceases to splice Xbp-1 mRNA. Instead, IRE1α mediates degradation of anti-Casp2 miRNAs through RIDD. IRE1α-mediated degradation of anti-Casp2 miRNAs leads to activation of the apoptotic initiator Casp2 and subsequently triggers mitochondrion-dependent apoptosis.
Figure 3. Updated model of inositol-requiring enzyme 1 alpha (IRE1α) and PERK signaling in cell fate determination during endoplasmic reticulum (ER) stress. Unfolded protein response (UPR) signaling aimed at cell survival is considered an adaptive response during ER stress. Under irremediable ER stress, the UPR represses the adaptive response and triggers an apoptotic response. IRE1α and PERK are two ER stress sensors that decrease ER protein-folding demand through mRNA decay and translational inhibition, respectively. Both PERK and IRE1α signaling appear to persist throughout ER stress. IRE1α differentially triggers diverse UPRs according to need. In the adaptive phase, to increase protein-folding capacity, IRE1α-mediated Xbp-1 mRNA splicing is activated for transcriptional regulation of UPR target genes. In a transition phase between the adaptive and apoptotic responses, the signaling mediated by IRE1α–Xbp-1 is attenuated. In parallel, IRE1α increases the intensity of the ER stress through mRNA decay of selective UPR target genes, including ER chaperones. During the apoptotic phase, IRE1α–Casp2 signaling is activated to initiate cell death.

the cleavage sites are relatively divergent [49,54]. This suggests that the cleavage mechanisms are likely to be conserved, whereas the flanking sequence determines the specificity of substrate recognition. This scenario would support the hypothesis that IRE1α adjusts its RNase substrate specificity to activate diverse UPRs. The flexibility of IRE1α to target different substrates might rely on combinations of phosphorylation status, conformational changes, and physical associations with IRE1α regulators. Because alterations of IRE1α substrate specificity lead to opposite cell fates [50], further understanding of IRE1α substrate preferences will reveal how IRE1α coordinates cellular homeostasis to determine cell fate under ER stress. Currently, target switching of RIDD has been reported only in animals. Therefore, to gain a deeper understanding of the evolution of the UPR in eukaryotes, further studies are needed to determine whether similar mechanisms exist in yeast and plants.

**Plant IRE1 in the ER stress response and cell fate determination**

Despite the conservation of IRE1 among eukaryotes, divergent IRE1-dependent regulatory events have also been observed between plants and mammals. These evolutionarily divergent mechanisms are likely to be the reason for the different ER stress and cell fate phenotypes observed between plants and mammals. Unlike mammalian IRE1 isoforms, the two Arabidopsis IRE1 isoforms are expressed ubiquitously with a limited tissue-specific expression pattern [55,56]. There is no significant defect of the UPR in single mutants of Arabidopsis IRE1A or IRE1B, whereas Arabidopsis ire1 double mutants display compromised ER stress tolerance and a UPR activation phenotype [17,18]. These observations indicate that the two Arabidopsis IRE1 homologs share partially overlapping function during the UPR. Evidence for established, dominant, or specific roles of individual Arabidopsis IRE1 isoforms during the UPR and cell fate regulation need to be further elucidated. Notably, it is experimentally undetermined whether viable Arabidopsis ire1b are knockouts or partial loss-of-function mutants. Failure to recover a homozygous plant of putative ire1b knockout hints that Arabidopsis IRE1B might be an essential gene similar to mammalian IRE1α [57]. Interestingly, although mammalian IRE1α is essential for the UPR in goblet cells, in other cell types there is no detectable defect in UPR target gene induction in a mammalian ire1 double mutant, probably due to partially overlapping function with ATF6 and PERK [46,58]. By contrast, although two functional homologs of ATF6, bZIP28 and bZIP17, exist in Arabidopsis [33] (Figure 1), Arabidopsis ire1 double mutants exhibit a dramatic reduction of UPR target gene activation [17,18]. These data indicate that the UPR is partially diversified between mammals and plants. Nonetheless, similar IRE1 features are also observed between plants and mammals. For instance, ire1 and xbp-1 mutants display differential phenotypes despite both being essential genes. Likewise, mutants of the Arabidopsis IRE1 splicing target bZIP60 show ER stress tolerance comparable with wild type plants as opposed to Arabidopsis ire1 double mutants [17], supporting the hypothesis that the function of Arabidopsis IRE1 is not restricted to unconventional splicing like mammalian IRE1.

Interestingly, mutations of IRE1 in plants and mammals lead to opposite effects in ER stress-induced cell death phenotypes [17,18,32]. ire1a−/− mouse embryonic fibroblasts (MEFs) exhibit a greater survival rate than Ire1α+/+ MEFs under ER stress, supporting the suggestion that mammalian IRE1 is an apoptosis executioner. By contrast, Arabidopsis ire1 double mutants display compromised ER stress tolerance, instead of a greater survival rate [17,18]. Consistently, DNA fragmentation and ion leakage are enhanced in the Arabidopsis ire1 double mutant during ER stress [32], suggesting that plant IRE1 might not function as an apoptosis executioner like its mammalian counterpart. Nevertheless, it cannot be
excluded that the differences are related to dissimilar experimental settings; mammalian UPR research is mostly conducted in cell culture, whereas intact organisms are used in plant UPR studies. Moreover, except for potential roles in protein-loading reduction under ER stress [32], the biological significance of plant RIDD in cell fate determination is unknown. Further experimental validation will reveal whether plant RIDD could process multiple substrates to control cell fate decisions, similar to that seen in mammals.

**Shared components of the UPRosome and apoptosis**

IRE1α activation is tightly controlled by its interacting protein complex, termed the UPRosome [15]. Most UPRosome components are involved in apoptosis, supporting the suggestion that intense crosstalk exists between IRE1α activity and apoptosis activation (Table 1). Specifically, although the UPRosome comprises multiple components, loss-of-function mutation of a single component, such as PARP16, Bi-1, Aip-1, PTP-1B, NMHCII, Jab1, Nck1, or Ask1, is sufficient to alter IRE1α splicing activity or apoptosis activation [59–66] (Table 1). Moreover, IRE1α-interactor mutants displaying either elevated or declined IRE1α splicing activity can show enhanced apoptosis, indicating that a precise level of activation of IRE1α splicing is important for cell survival [59–66]. This further suggests that IRE1α activation is controlled by a signaling network that maintains a delicate equilibrium of adaptive and apoptotic responses. A subtle imbalance of the equilibrium could disturb cellular homeostasis and thus alter cell fate determination [59–76]. Furthermore, the observation that a single mutation of the UPRosome leads to significant defects in IRE1α signaling hints that IRE1α is differentially regulated in a context-specific manner (Table 1). Because UPRosome analyses are conducted under various conditions, systematic and comparable analyses of UPRosome members will connect each hub and thus give a clearer view of the IRE1α signaling network.

**IRE1 sensing mechanisms**

ER stress-sensing mechanisms are intensively studied in yeast and animals [77]. The ER stress sensors are silent through physical association with BiP, the most abundant ER-resident chaperone. Dissociation with BiP or interaction with unfolded proteins is the major trigger of IRE1 activation. Yeast IRE1 is activated through association with unfolded proteins rather than dissociation with BiP [78]; however, the physical interaction of BiP is a fine-tuning mechanism to ensure that yeast IRE1 is appropriately activated [79]. Unlike yeast IRE1, the activation mechanisms of mammalian IRE1α rely on dissociation with BiP as opposed to a direct interaction with unfolded proteins [80]. The differences in activation mechanisms between yeast and mammalian IRE1α can be partially explained by the dissimilarity in protein structure within the sensor domain [15]. Surprisingly, a recent study revealed that mammalian IRE1β tends to interact with unfolded proteins like yeast IRE1 and is unable to associate with BiP [81]. Accordingly, it is possible that, like yeast IRE1, binding of unfolded proteins is the primary trigger of mammalian IRE1β activation. Despite intense studies in mammals and yeast, plant IRE1 sensing mechanisms are completely undefined. Further structural and functional analyses of plant IRE1 will be instrumental in revealing ER stress-sensing mechanisms in plants.

**Cell type-specific sensing mechanisms: the role of mammalian IRE1β**

How cellular homeostasis is maintained in a cell type-specific manner is a fundamental question of cell biology. It has been recently shown that IRE1β is essential for the UPR specifically in goblet cells [46]. In goblet cells, IRE1β is dispensable for Xbp-1 splicing and BiP induction. Instead, IRE1β mutation leads to enhanced ER stress intensity evidenced by higher levels of Xbp-1 splicing and BiP induction. Moreover, IRE1β−/− mice display a distended ER phenotype, potentially due to overaccumulation of

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**Table 1. Interacting proteins of IRE1α**

<table>
<thead>
<tr>
<th>IRE1α interactors</th>
<th>Function of IRE1α interactors</th>
<th>Observed phenotype of loss-of-function mutations</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP16</td>
<td>Poly ADP-ribose polymerase</td>
<td>Decreased Xbp-1 splicing/increased cell death</td>
<td>[59]</td>
</tr>
<tr>
<td>Bi-1</td>
<td>Anti-apoptotic protein</td>
<td>Increased Xbp-1 splicing/increased cell death</td>
<td>[60]</td>
</tr>
<tr>
<td>Aip-1</td>
<td>Transducer of apoptotic signaling</td>
<td>Decreased Xbp-1 splicing/decreased cell death</td>
<td>[61]</td>
</tr>
<tr>
<td>Ptp-1b</td>
<td>Protein tyrosine phosphatase 1B</td>
<td>Decreased Xbp-1 splicing/decreased cell death</td>
<td>[62]</td>
</tr>
<tr>
<td>NMHCII</td>
<td>Myosin cytoskeleton</td>
<td>Decreased Xbp-1 splicing/compromised IRE1α foci formation</td>
<td>[63]</td>
</tr>
<tr>
<td>Bax/Bak</td>
<td>Pro-apoptotic protein</td>
<td>Decreased Xbp-1 splicing/impaired IRE1α oligomerization</td>
<td>[67]</td>
</tr>
<tr>
<td>Bim/Puma</td>
<td>Pro-apoptotic protein</td>
<td>Decreased Xbp-1 splicing and UPR target genes activation</td>
<td>[76]</td>
</tr>
<tr>
<td>Jab1</td>
<td>Apoptosis-related protein</td>
<td>Decreased Xbp-1 splicing and UPR target genes activation</td>
<td>[64]</td>
</tr>
<tr>
<td>Nck1</td>
<td>SH2/SH3 domain containing adaptor</td>
<td>Decreased cell death</td>
<td>[65]</td>
</tr>
<tr>
<td>Ask1</td>
<td>Apoptosis signal-regulated kinase</td>
<td>Decreased cell death/altered JNK activation</td>
<td>[66]</td>
</tr>
<tr>
<td><strong>IRE1α interactors</strong></td>
<td><strong>Function of IRE1α interactors</strong></td>
<td><strong>Observed phenotype of induction, overexpression, or inhibition</strong></td>
<td><strong>Refs</strong></td>
</tr>
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<td>Traf2</td>
<td>Tumor necrosis factor</td>
<td>Decreased JNK activation by expression of dominant-negative Traf2</td>
<td>[72]</td>
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<tr>
<td>Jik</td>
<td>JNK inhibitory kinase</td>
<td>Increased JNK activation by overexpression of JIK</td>
<td>[68]</td>
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<tr>
<td>Hsp90</td>
<td>Heat shock protein</td>
<td>Decreased IRE1α protein stability by treatment of HSP90 inhibitors</td>
<td>[71]</td>
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<tr>
<td>Usp14</td>
<td>Ubiquitin specific peptidase</td>
<td>Increase activity of ERAD by small interfering RNA silencing of Usp14</td>
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<tr>
<td>SYVN1</td>
<td>E3 ubiquitin ligase/anti-apoptotic factor</td>
<td>Increased IRE1 ubiquitination and degradation by coexpression of IRE1 and SYVN1</td>
<td>[73]</td>
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<tr>
<td>Hsp72</td>
<td>Heat shock protein</td>
<td>Increased Xbp-1 splicing/decreased cell death by induction of HSP72</td>
<td>[70]</td>
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<tr>
<td>Rack1</td>
<td>Scaffold protein for activated protein kinase</td>
<td>Decreased IRE1α phosphorylation by overexpression of RACK1</td>
<td>[75]</td>
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Mucin2 (MUC2), the most prominent protein secreted from goblet cells. This suggests that IRE1α controls MUC2 expression in goblet cells. Thus, IRE1β mutation leads to MUC2 overload in the ER and in turns triggers ER stress [46]. RIDD was proposed to be the mechanism underlying IRE1β regulation of MUC2 levels in goblet cells [46]. The cell type-specific target of IRE1β provides a molecular explanation of how the UPR maintains a dynamic and specific secretory ability in multicellular organisms. Consistent with the notion that unfolded proteins trigger IRE1β activation, IRE1β might interact physically with specific types of unfolded protein. In the case of goblet cells, IRE1β might specifically monitor the MUC2 level in the ER and adjusts its loading into the ER through RIDD. Based on this scenario, mammalian ER stress sensors might distinguish the type of unfolded protein accumulated in the ER and trigger differential UPR signaling. More specifically, if the unfolded proteins are dispensable for cell survival, ER stress sensors could repress the expression of unfolded proteins through RNA decay or translational repression. Conversely, if unfolded proteins are essential for cellular function, the UPR might preferentially augment the expression of chaperones to recover the production of unfolded proteins. Although ER stress duration and intensity are considered major factors in the apoptosis threshold, the type of misfolded protein might be also critical for the determination of UPR signaling outputs.

**Sensing mechanisms beyond protein-folding homeostasis**

Emerging evidence shows that IRE1 monitors cellular homeostasis beyond sensing unfolded protein accumulation. For instance, CRY1/CRY2-mediated circadian rhythm regulates IRE1α activity in the liver [82], suggesting that IRE1α coordinates ER function to cope with circadian-related physiological processes. These observations provide a link between the IRE1α-dependent UPR, circadian regulation, and liver metabolic processes. More importantly, because circadian rhythm has a substantial influence on UPR activation, time-course studies of the UPR will require diligent experimental design and appropriate controls to avoid biases. Recently, lipid homeostasis was proven to impact UPR activation through an unconventional sensing mechanism, because the unfolded protein-sensing domain of IRE1α and PERK is dispensable for lipid-dependent UPR activation [83]. Together, these observations support the suggestion that the UPR perceives physiological and cellular signaling beyond ER protein-folding homeostasis. Although it is unclear whether plant IRE1 senses signaling beyond protein-folding capacity, an Arabidopsis ire1 double mutant displaying a root-specific phenotype under unstressed conditions hints that plant IRE1 also integrates physiological signals to maintain specific secretory activity [17]; however, this hypothesis awaits experimental validation.

**Concluding remarks**

Significant progress in defining IRE1 mechanisms has been achieved. We now know that IRE1 activities are coordinated at a systemic level to cope with dynamic secretion activity. Although in vitro experimental systems and conditional IRE1 induction approaches have made groundbreaking discoveries in basic UPR knowledge [49,50,84–87], we remain far from a comprehensive understanding of the UPR in intact organisms. The lethality of the mammalian IRE1α mutant represents a challenge to gaining insights into IRE1 function in vivo. By contrast, the viability of plant IRE1 mutants enables in vivo analyses to reveal its roles in organ growth, pathogen defense, and abiotic stress responses [17,33,88,89]. Moreover, with the ease of building high-order plant mutants, in vivo phenotypic analyses show that Arabidopsis IRE1 and a conserved component of the G protein complex display a synergistic effect in both plant UPR activation and growth regulation. This study underscores the building of UPR networks in intact organisms using plants as a model system [17]. With more systematic and quantitative studies of the UPR in vivo, there are significant findings ahead that will decipher dynamic UPR maps close to a genuinely physiological scenario.

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