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The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation

Peter Walter¹ and David Ron²

The vast majority of proteins that a cell secretes or displays on its surface first enter the endoplasmic reticulum (ER), where they fold and assemble. Only properly assembled proteins advance from the ER to the cell surface. To ascertain fidelity in protein folding, cells regulate the protein-folding capacity in the ER according to need. The ER responds to the burden of unfolded proteins in its lumen (ER stress) by activating intracellular signal transduction pathways, collectively termed the unfolded protein response (UPR). Together, at least three mechanistically distinct branches of the UPR regulate the expression of numerous genes that maintain homeostasis in the ER or induce apoptosis if ER stress remains unmitigated. Recent advances shed light on mechanistic complexities and on the role of the UPR in numerous diseases.

Secreted and membrane proteins fold and mature in the lumen of the endoplasmic reticulum (ER) before they are delivered to other compartments in the endomembrane system, displayed on the cell surface, or released extracellularly. A collection of phylogenetically conserved signaling pathways, collectively termed the unfolded protein response (UPR), monitors conditions in the ER, sensing an insufficiency in the ER's protein-folding capacity (and hence the threat of misfolding) and communicates this information regarding the status of the ER lumen to gene expression programs of eukaryotic cells [reviewed in (1)]. UPR activation increases ER abundance to match needs by mediating expansion of the ER membrane (2) and populating the expanded organelle space with newly synthesized protein-folding machinery. This long-term, largely transcriptional control is accompanied by mechanisms that transiently decrease the flux of protein entering the ER. As such, the UPR is a paradigm for countless other feedback loops that establish and maintain homeostasis.

Progress in cell biology is most beautifully revealed when complex cellular events become understood at the level of the molecular machines that orchestrate them. The UPR is one of these examples, where detailed molecular description has begun to elucidate how a eukaryotic cell regulates the abundance of its ER. The explosion of mechanistic knowledge has opened doors to entirely unanticipated discoveries concerning how the UPR is intricately integrated with other aspects of cell physiology to sustain homeostatic balance [reviewed in (3)].

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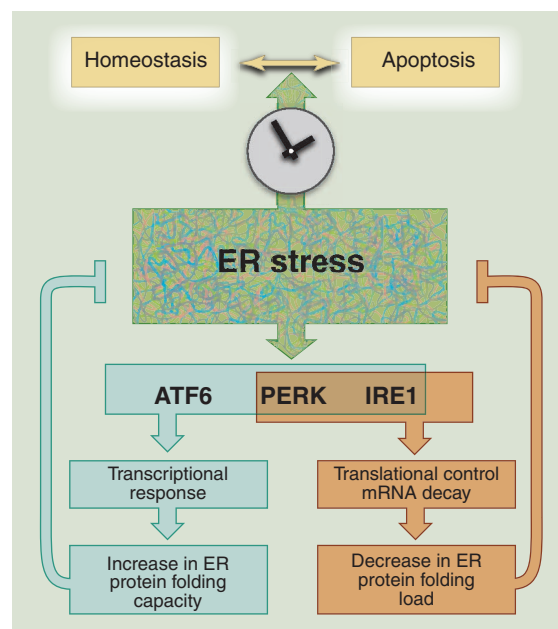


Fig. 1. A simplified wiring diagram of the core elements of the UPR signaling network. ER stress activates the stress sensors ATF6, IRE1, and PERK, representing the three branches of the UPR. Activation of each sensor produces a transcription factor [ATF6(N), XBP1, and ATF4, respectively] that activates genes to increase the protein-folding capacity in the ER. IRE1 (via RIDD) and PERK (via eIF2 α phosphorylation) also decrease the load of proteins entering the ER. Both outcomes work as feedback loops that mitigate ER stress. If cells cannot reestablish homeostasis but continue to experience prolonged and unmitigated ER stress (depicted by the timer), they apoptose.

Virtually all signaling proteins that a eukaryotic cell uses to communicate with its environment are assembled in the ER. They transmit and receive information crucial to the health of the organism, such as informing cells when to divide,

migrate, differentiate, or die. Checkpoints to ensure that these components are assembled with high fidelity are to be expected; without such quality control, chaos would ensue. One of the primary functions of the ER is to exert such quality control on the proteins it makes: Only properly folded proteins are packaged into ER exit vesicles and allowed to move onward to be displayed on the cell surface (4, 5). Improperly folded proteins are retained in the ER and delivered for proteasomal degradation after retrotranslocation into the cytosol, a process called ER-associated degradation (ERAD) (6). ERAD is essential in cells that cannot induce the UPR (7), hinting at the importance of continual removal of polypeptides that fail to reach their native state.

Prolonged activity of the UPR, an indication that ER stress cannot be mitigated and homeostasis cannot be reestablished, correlates with cell death [reviewed in (8)]. This suggests that the commitment to apoptosis in this context

may have evolved to protect the organism from rogue cells that lack the capacity to ascertain the fidelity of their signaling components. A life-or-death decision, based on an assessment of whether ER stress can be mitigated in a timely fashion, nicely explains the UPR's central role in numerous human diseases. When homeostasis fails, the UPR can serve as an apoptotic executor that kills cells that would be beneficial, or as a cytoprotector that safeguards rogue cells to the detriment of the organism. Examples in the first category include protein-misfolding diseases such as retinitis pigmentosa, an inherited form of blindness in which the retina degenerates by apoptotic cell death when a misfolded mutant rhodopsin is produced during retinal development (9). Another such example is type II diabetes, in which pancreatic beta cells are compromised by excessive demand for insulin production (10). The second category is exemplified by enveloped virus infections that can exploit the UPR to increase the capacity of the ER to assist in viral replication (11). Similarly, certain types of cancer—especially those that arise in secretory tissues, such as multiple myeloma—use the cytoprotective role of the UPR to sustain their rapid growth (12, 13).

Given the dichotomy in outcomes of UPR activation, it remains unclear whether a window exists in which manipulation of the UPR can be harnessed therapeutically. Thus, it is important to develop a precise understanding of the molecular mechanism of signal trans-

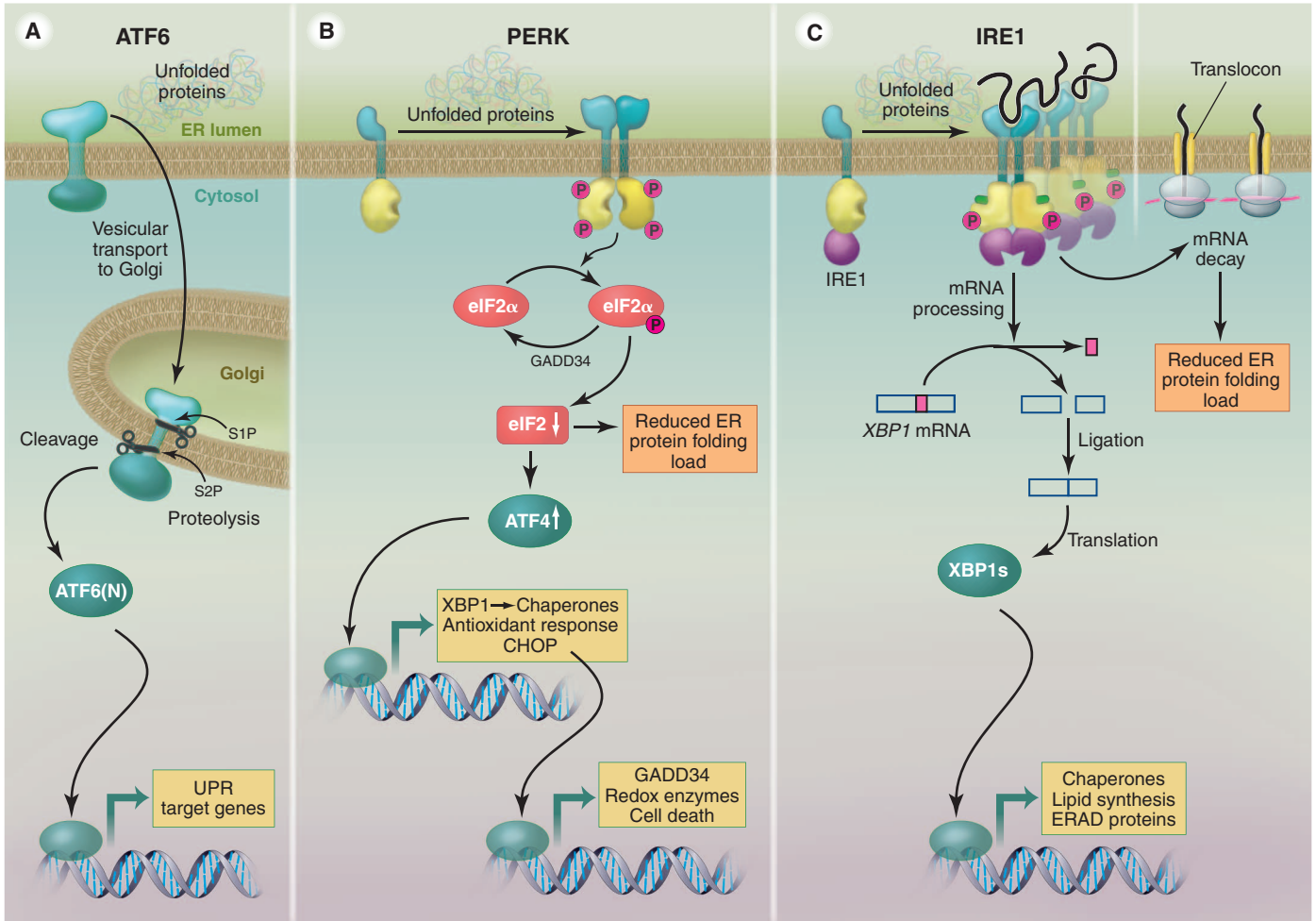


Fig. 2. (A to C) The three branches of the UPR. Three families of signal transducers (ATF6, PERK, and IRE1) sense the protein-folding conditions in the ER lumen and transmit that information, resulting in production of bZIP transcription regulators that enter the nucleus to drive transcription of UPR target genes. Each pathway uses a different mechanism of signal transduction: ATF6 by

regulated proteolysis, PERK by translational control, and IRE1 by nonconventional mRNA splicing. In addition to the transcriptional responses that largely serve to increase the protein-folding capacity in the ER, both PERK and IRE1 reduce the ER folding load by down-tuning translation and degrading ER-bound mRNAs, respectively.

duction in the UPR and to develop compounds that can selectively modulate discrete steps of the pathway.

The Three UPR Signal Transducers

Three principal branches of the UPR have been identified (1) (Fig. 1). The branches operate in parallel and use unique mechanisms of signal transduction. Each branch is defined by a class of transmembrane ER-resident signaling components: IRE1 (inositol requiring enzyme 1), PERK [double-stranded RNA-activated protein kinase (PKR)-like ER kinase], and ATF6 (activating transcription factor 6). The IRE1 branch is the most conserved and sole branch of the UPR in lower eukaryotes (14). Evolution later added the PERK and ATF6 branches to metazoan cells. The UPR branches are differently represented in different cell types; moreover, there are multiple genes encoding ATF6 family members and two IRE1 paralogs in mammalian cells, hinting at tissue and cell type specialization of the UPR that remains poorly understood. Activation of each

branch leads to the production of b-ZIP transcription factors, which work alone or together to activate UPR target genes.

ATF6 is a transcription factor that is initially synthesized as an ER-resident transmembrane protein bearing a large ER-luminal domain (Fig. 2A). Upon accumulation of unfolded proteins, it is packaged into transport vesicles that pinch off the ER and deliver it to the Golgi apparatus (15). There, it encounters two proteases, S1P and S2P (site-1 and site-2 protease), that sequentially remove the luminal domain and the transmembrane anchor, respectively (16, 17). The liberated N-terminal cytosolic fragment, ATF6(N), then moves into the nucleus to activate UPR target genes. Among ATF6's targets are prominent ER-resident proteins involved in protein folding, such as BiP (a chaperone of the heat shock protein HSP70 family), protein disulfide isomerase, and glucose-regulated protein 94 (GRP94; a chaperone of the Hsp90 family). ATF6 processing resembles the mechanism by which sterol response element binding protein (SREBP), the transcrip-

tion factor that controls sterol biosynthesis, is regulated in mammalian cells and uses the same proteases (18). Whereas the mechanism of SREBP control at the level of ER exit is well understood, little is known about how ATF6 responds to ER stress. Its ER-luminal domain shows no sequence homology to other proteins. ATF6 associates with BiP, and BiP release under conditions of ER stress may contribute to its activation. The ATF6 luminal domain also contains intra- and intermolecular disulfide bonds that may monitor the ER environment as redox sensors.

The second branch of the UPR is mediated by PERK, an ER-resident transmembrane kinase (Fig. 2B). When activated upon sensing ER stress, PERK oligomerizes and phosphorylates itself and the ubiquitous translation initiation factor eIF2 α , indirectly inactivating eIF2 and inhibiting mRNA translation. In this way, PERK helps reduce the flux of protein entering the ER to alleviate ER stress. However, some mRNAs containing short open reading frames in their 5'-untranslated regions are preferentially translated

when eIF2 is limiting. One of these encodes the transcription factor ATF4, whose translation is thus induced. Two important target genes driven by ATF4 are CHOP (transcription factor C/EBP homologous protein) and GADD34 (growth arrest and DNA damage-inducible 34). CHOP is a transcription factor that controls genes encoding components involved in apoptosis. Thus, the PERK branch of the UPR is strongly protective at modest levels of signaling but can contribute signals to cell death pathways. This dualism is likely played out at the level of phosphorylated eIF2 α and is exemplified by the effects of manipulation of its specific phosphatases. GADD34 encodes a PERK-inducible regulatory subunit of the protein phosphatase PP1c that counteracts PERK by dephosphorylating eIF2 α . Selective inhibition of the GADD34-PP1c complex, either by a small molecule or deletion of GADD34, protects cells against ER stress by prolonging low-level eIF2 α phosphorylation (19, 20). The importance of such balanced regulation of eIF2 α dephosphorylation is emphasized by the lethal consequences of deletion of the constitutive eIF2 α phosphatase, CREP, when GADD34 is compromised (20, 21).

IRE1 defines the third and, because of its presence in yeast, best-studied branch of the UPR (Fig. 2C). It is a bifunctional transmembrane kinase/endoribonuclease that uses a unique mechanism of nonconventional mRNA splicing to transmit the UPR signal. Its ribonuclease (RNase) function is activated by conformational changes following lateral IRE1 oligomerization in the ER membrane. When activated, IRE1 cleaves the mRNA encoding a UPR-specific transcription factor, called XBP1 (X-box binding protein 1) in metazoans, in two specific positions, excising an intron. The severed exons are then ligated (by tRNA ligase in yeast and by one or more yet-undiscovered enzymes in mammalian cells), giving rise to a spliced mRNA that is translated to the active forms of the transcription factor XBP1^s (the superscript indicates that it is the product of the spliced mRNA). In yeast, IRE1 activity drives the entire UPR gene expression program, whereas in metazoans there appears to be considerable redundancy between UPR-induced transcription factors. Nonetheless, XBP1^s appears to have a special role in regulating lipid biosynthetic enzymes and ER-associated degradation components, as well as in promoting the development of an elaborate ER that is characteristic of active secretory cells (22).

Molecular Insight into IRE1 Activation

Structural and biophysical experiments have provided a detailed view of IRE1 activation. RNase activation proceeds from inactive monomers that assemble into back-to-back dimers (23), which further stack into higher-order oligomers (24). During activation, IRE1 autophosphorylates, perhaps in a front-to-front interaction between IRE1 monomers; such a conformation would favor the activating trans-autophosphorylation event but

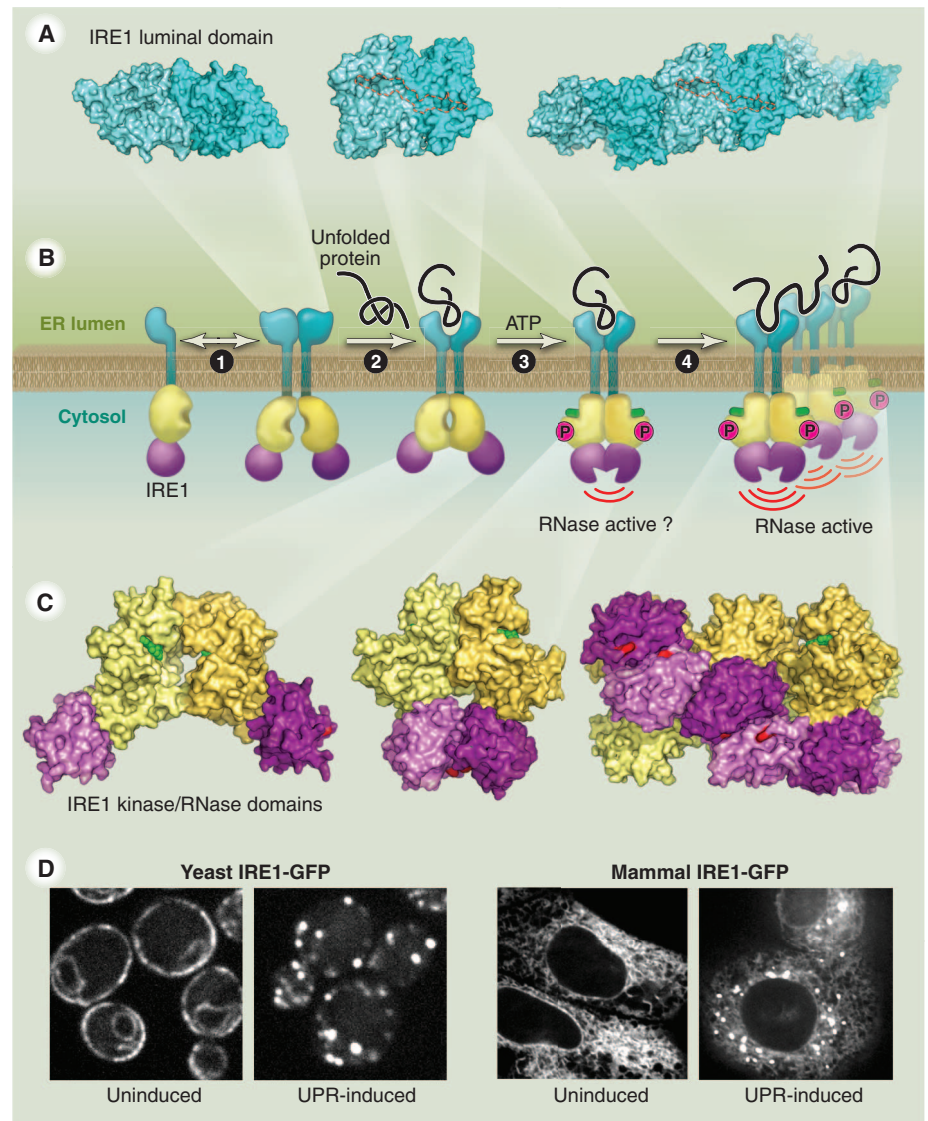


Fig. 3. A speculative model of IRE1 activation. The figure depicts multiple structures of the IRE1 luminal ER stress-sensing domain (A) and the IRE1 cytosolic kinase and RNase domains (C), aligned in a hypothetical sequence (B) by which IRE1 may be activated in response to an accumulation of unfolded proteins in the ER lumen. IRE1 protomers (step 1) engage homotypically in the plane of the membrane. ER luminal domains may form a closed dimer in which the proposed peptide-binding groove is occluded and further oligomerization is prevented (40). In response to protein misfolding (step 2), the luminal domain rearranges, allowing unfolded protein binding (proposed peptide-binding groove outlined in red) and further oligomerization (38), leading to front-to-front interactions of the kinase/RNase domains on the other, cytosolic side of the ER membrane. Such interactions are observed in crystals on the nonphosphorylated human IRE1 and may correspond to a pre-autophosphorylation complex (25). After trans-autophosphorylation, IRE1 forms back-to-back dimers (step 3) (24) that stack into oligomers (step 4) (23). The crystallized IRE1 kinase domains (shown in gold) contain either a bound adenosine diphosphate–Mg complex (23) or a kinase inhibitor (24) shown in green. The RNase active site is located in the cleft between two RNase monomers (shown in purple with the catalytic histidines in red) as arranged in the IRE1 back-to-back dimer and oligomer. PDB access numbers: 2HZ6 and 2BE1 for structures shown in (A); 3P23, 2RIO, and 3FBV for those shown in (C). (D) Clusters of activated, oligomeric IRE1 are visible by fluorescence microscopy as discrete foci in yeast (*Saccharomyces cerevisiae*) and mammalian (HEK293) cells.

leave the dimeric RNase site unassembled (25). Trans-autophosphorylation may continue between stacked dimers in the IRE1 oligomer (24) (Fig. 3).

Phosphorylation of IRE1 on its activation loop, as with other protein kinases, enhances nucleo-

tide binding to its kinase active site; however, the phosphorylation state of IRE1 is likely to modulate its activity in other ways as well. In the structure of the IRE1 oligomer, a small number of phosphates form stabilizing salt bridges to

adjacent protomers, suggesting a role for phosphorylation in IRE1 activation (24). In contrast to PERK and all other protein kinases that conventionally propagate a signal via phosphoryl-transfer reactions, the kinase activity of IRE1 can be entirely bypassed. In yeast, IRE1 mutants with inactivated kinase activity still oligomerize in response to an accumulation of unfolded protein in the ER and mediate the mRNA splicing reaction, albeit with slightly reduced activity. Surprisingly, such mutants are much delayed in turning off the splicing reaction after resolution of ER stress. Failure to properly inactivate IRE1 inappropriately prolongs UPR signaling and reduces cell survival under UPR-inducing conditions (26, 27). Thus, autophosphorylation is a critical feature of the UPR homeostatic feedback loop. Phosphates added early may help to stabilize IRE1 oligomers as they form and open up the active site to promote binding of further activating ligands. Phosphates added later may destabilize oligomers, perhaps simply by building up charge repulsion in the IRE1 oligomer or by providing binding sites for other factors that aid in oligomer disassembly. Such a mechanism might promote a dynamic equilibrium between phosphorylated IRE1 embedded in the active oligomers and hyperphosphorylated IRE1 prone to dissociate from the active oligomers.

There is increasing evidence that the threshold concentration that triggers IRE1 activation can be modulated by various means. For example, small molecules that bind to IRE1's kinase active site can be inhibitors or activators of various potencies (28). Binding of these compounds is thought to shift the equilibrium between two conformational states that are conserved among protein kinases: "αC-helix in" and "αC-helix out." In the first of these conformations, IRE1 is prone to oligomerize and activate; in the second, it is inactive. The kinase domain of IRE1 thus acts as a conformational module, and occupancy with ligands can modulate the activation threshold. This provides a means for IRE1 to be regulated by nucleotides (or perhaps other metabolites that engage its nucleotide-binding pocket). Yeast IRE1 has a second ligand binding site at the dimer interface through which oligomerization and activity can be modulated, so far only in vitro (29).

IRE1's Interaction with Substrates

Recent work has drawn attention to the mechanisms that target the *XBPI* mRNA substrate to IRE1. In budding yeast, the *HAC1* mRNA (the yeast XBP1 ortholog) contains a targeting signal in its 3'-untranslated region that is required and sufficient to localize the mRNA to activated IRE1 (30). By contrast, mammalian XBP1^u, the protein translated from unspliced *XBPI* mRNA, contains an internal hydrophobic stretch in its C-terminal region that acts as a signal sequence to bring the XBP1^u-translating polyribosome to the ER membrane (31). In plants, the XBP1 homolog bZIP60 is also translated from the un-

spliced mRNA and is targeted to the ER membrane as an integral membrane protein (32). In both cases, splicing shifts the open reading frame so that the hydrophobic targeting sequences are no longer translated, resulting in the production of the soluble transcription factors. Thus, intriguing parallels exist between bZIP60 and ATF6, where transcription factors are produced upon UPR induction from resident ER membrane proteins by mRNA splicing or proteolysis, respectively.

The cytoplasmic kinase/RNase domain of yeast IRE1 displays strongly cooperative activation kinetics, indicating that more than two IRE1 molecules must assemble to form a fully active enzyme (24). Oligomerization can be visualized in the light microscope by following fluorescently tagged IRE1 fusion proteins (Fig. 3D) (30, 33, 34), which upon UPR activation cluster dynamically into discrete foci in the ER membrane in close enough molecular proximity to allow fluorescent energy transfer between fluorophores (35). One model based on a crystal structure of the active, oligomeric form of IRE1 poses that protein interfaces, which form when IRE1 dimers stack in an oligomeric assembly, stabilize the RNase active site, providing an intuitive view of how oligomerization and enzymatic activation may be coupled. Because of its cooperative nature, IRE1 RNase activates abruptly upon reaching a critical threshold concentration (24), which lends switch-like properties to IRE1's cytosolic module. In the context of the cell, the signal of many IRE1 modules producing such binary output must be integrated to produce a graduated response that reflects the strength of the input signal and is useful for homeostatic control. Such integration could occur simply by summing up signals from multiple locations in the ER, in effect "counting" the number of active IRE1 clusters at any one time.

Unfolded Protein Sensing and Alternative Modes of UPR Activation

To sense unfolded proteins in the ER lumen, UPR signaling proteins must be subservient to

their luminal sensor domains. However, all proteins enter the ER in an unfolded state. Thus, the activation thresholds of IRE1, PERK, and ATF6 must be properly tuned. Early work suggested that binding to BiP retains IRE1 and PERK in a monomeric, inactive state, and that competition by unfolded proteins for BiP favors its dissociation from the luminal domains, allowing spontaneous oligomerization of PERK and IRE1 (1). However, subsequent work in yeast showed that IRE1 mutants that no longer bind BiP in a measurably regulated manner activate their UPR efficiently (35, 36), indicating that IRE1 regulation can proceed independently of regulated BiP release. An alternative model suggests that IRE1 binds to unfolded proteins directly and that these serve as activating ligands. This direct binding model is supported by the crystal structure of the yeast IRE1 luminal domain, which reveals a major histocompatibility complex (MHC)-like architecture containing a groove poised for peptide binding (37). Recent evidence that IRE1 binds to unfolded proteins and that extended peptides trigger oligomerization of the IRE1 luminal domain provides further support for the idea that IRE1 directly senses unfolded proteins (38, 39). The mammalian IRE1 luminal domain crystallizes in a different conformation with a closed binding groove (40), which suggests that conformational changes that stabilize the open conformation upon peptide binding may trigger oligomerization and thus lead to IRE1 activation (Fig. 3A). Rather than providing the switch that activates the UPR, the interaction of the IRE1 (and, by extension, PERK) luminal domain with BiP may serve a subtler role as a buffer for monomers, thereby stabilizing at an appropriate level the concentration of IRE1 monomers available for activation by unfolded protein ligands (35).

Although unfolded protein recognition is generally regarded as the primary mode of UPR activation, there is increasing evidence that the luminal domain does not always govern IRE1 activation. Surprisingly, IRE1 in which the luminal domain is eliminated and replaced by a

Box 1. Major unsolved questions.

1. What is the basis for toxicity of unfolded or misfolded proteins in the ER?
2. How can we define and experimentally measure unfolded protein burden in the ER? What fraction of ER chaperones is engaged with unfolded proteins?
3. What is the ultrastructure of active IRE1 and PERK in the ER membrane? What other components are part of these signaling platforms?
4. Are there effectors of the IRE1 kinase domain other than IRE1 itself?
5. How is ATF6 activated?
6. How can we rationalize the division of target genes among the three UPR branches? What was the evolutionary drive toward this specialization?
7. Are RIDD- and PERK-mediated translational control spatially restricted?
8. What mechanistic details distinguish IRE1-mediated mRNA splicing and RIDD?
9. Is there a therapeutic window in which manipulation of the UPR can be beneficial in treating human disease?
10. How do IRE1, PERK, and ATF6 sense membrane aberrancy?

leucine zipper (thus rendering it a constitutive dimer) remains strongly inducible by changes in the lipid composition (41). It is possible that the artificial dimerization provides seeds that poise IRE1 for oligomerization upon perturbations in the membrane environment. Similarly, ATF6 (but not IRE1) is selectively activated by overexpression of an ER tail-anchored protein that contains only a few amino acids in the ER lumen (42). The transcriptional profile resulting from this activation event is qualitatively distinct from that obtained upon UPR activation by unfolded proteins, which reinforces the notion that preferential activation of individual UPR branches modulates the response.

Another instance in which IRE1 signaling may not rely on ER-luminal sensing of unfolded proteins is IRE1 activation during B cell differentiation into plasma cells. Because of the massive amount of immunoglobulins secreted by plasma cells, the ER becomes highly amplified, and hence *XBPI*^s expression is required for this developmental step (22). Unexpectedly, UPR induction precedes a measurable ramping-up of immunoglobulin expression, which suggests that IRE1 activation in this case may be driven by a developmental switch rather than by overburdening the ER with a secretory load (43). Indeed, mutant B cells that make no immunoglobulin still induce IRE1 in response to differentiation signals (44). In this example, the UPR appears to act in an “anticipatory” mode, switched on by cellular cues that may not originate in the ER lumen, in contrast to the classical “reactive” mode that responds to conditions within the ER.

Nontranscriptional Aspects of UPR Regulation

IRE1-mediated splicing of *XBPI* mRNA is extraordinarily specific. In budding yeast, the orthologous *HAC1* mRNA stands out as the single identifiable IRE1 substrate, and no mRNAs other than *XBPI* are known to be spliced in an IRE1-dependent way in metazoans. This highly specific mode of mRNA engagement with IRE1 is distinct from a parallel mode of RNA cleavage in which diverse mRNAs are degraded in an IRE1-dependent fashion (45). This pathway, called RIDD (“regulated IRE1-dependent decay”), degrades ER-bound mRNAs in metazoans and may serve to limit protein influx and unfolded protein load into the ER lumen after prolonged UPR induction (Fig. 2C). It is thought that mRNAs are first nicked by the IRE1 endonuclease at sites that, by contrast to the well-conserved splice junctions in *XBPI* mRNA, do not display an identifiable consensus sequence, and hence may be relatively degenerate. The free ends thus generated are substrates for exonucleolytic decay by the cytosolic exosome. Weak activation of IRE1 attained by small molecules uncouples *XBPI* mRNA splicing from RIDD, raising the question of how IRE1 switches between specific and promiscuous modes of cleavage (46, 47). One possibility is that IRE1 assumes a qualitatively different state, perhaps governed by its kinase domain and ligands bound

to it. Alternatively, IRE1’s promiscuous mode may more simply reflect grades of RNase activity responsive to the strength and duration of underlying ER stress, perhaps mediated by the assembly of higher-order oligomers in which multiple RNA binding sites line up to promote avidity (24).

Both RIDD and the translational inhibition that occurs in response to eIF2 α phosphorylation by PERK have similar consequences in that they reduce the influx of proteins into the ER. Both mechanisms must be carefully controlled, because excessive activation is detrimental to cell survival. Reducing the load of proteins entering the ER must be balanced with the need to sustain sufficient synthesis of the protein-folding machinery itself and of all the other essential proteins that fold in the ER. Indeed, the similarities between RIDD and eIF2 α phosphorylation may extend further if we consider that both events are poised to exert local control: RIDD targets mRNAs that are in proximity to activated clusters of IRE1, whereas phosphorylation presumably targets eIF2 α molecules in proximity to activated PERK. We speculate that under normal cell growth conditions, subtle fluctuations in capacity and demand may be restricted to certain regions of the ER rather than affecting the entire organelle, and that localized activation of PERK and RIDD may allow spatially restricted fine-tuned homeostatic adjustments. This is in contrast to the global control by activation of transcriptional programs effected by the three UPR branches, which integrate signals across the cell. The altered gene expression resulting from their action in turn affects the cell as a whole.

Consequences of Sustained ER Stress

Cell-wide integration of UPR signaling is particularly important when cells make the decision to commit to apoptosis. It is unclear whether a single or multiple alternative mechanisms result in ER stress-induced cell death. One attractive possibility is that the three UPR branches provide opposing signals and that the relative timing of their induction shifts the balance between cytoprotection and apoptosis as unmitigated ER stress persists. IRE1 signaling, for example, attenuates upon prolonged ER stress (48), and, likewise, PERK signaling induces its own deactivation via GADD34 expression. Both pathways thus contain intrinsic timers that are likely to contribute to the life-or-death decision. The complexity of regulation and our challenge of deciphering it are further increased because the very components of the UPR, including IRE1, *XBPI*, PERK, and ATF6, are themselves transcriptionally controlled by the UPR. Moreover, apoptosis is only one possible outcome of chronic ER stress. Studies in chondrocytes, which abundantly secrete collagens, have shown that dedifferentiation away from a secretory cell phenotype may play a role in adaptation to chronic ER stress (49). This suggests that the pathogenic features of chronic ER

stress may be played out not only at the level of cell death but also at the level of altered cell function.

Conclusions

The role of the UPR is to protect cells against defects in protein folding in the ER. Because many mechanisms come into play, the cell carefully balances various means at its disposal to protect against proteotoxicity while also providing adequate protein synthesis to sustain fitness. Despite enormous progress in the field, many fundamental questions remain unanswered (Box 1). The potential impact of the UPR in many human diseases makes UPR signaling a promising target for therapeutic intervention.

References and Notes

1. D. Ron, P. Walter, *Nat. Rev. Mol. Cell Biol.* **8**, 519 (2007).
2. S. Schuck, W. A. Prinz, K. S. Thorn, C. Voss, P. Walter, *J. Cell Biol.* **187**, 525 (2009).
3. D. T. Rutkowski, R. S. Hegde, *J. Cell Biol.* **189**, 783 (2010).
4. S. M. Hurlbert, D. G. Bole, H. Hoover-Litty, A. Helenius, C. S. Copeland, *J. Cell Biol.* **108**, 2117 (1989).
5. L. Ellgaard, A. Helenius, *Nat. Rev. Mol. Cell Biol.* **4**, 181 (2003).
6. M. H. Smith, H. L. Ploegh, J. S. Weissman, *Science* **334**, 1086 (2011).
7. K. J. Travers *et al.*, *Cell* **101**, 249 (2000).
8. I. Tabas, D. Ron, *Nat. Cell Biol.* **13**, 184 (2011).
9. J. H. Lin, M. M. Lavail, *Adv. Exp. Med. Biol.* **664**, 115 (2010).
10. S. G. Fonseca, J. Gromada, F. Urano, *Trends Endocrinol. Metab.* **22**, 266 (2011).
11. B. Li *et al.*, *Virus Res.* **124**, 44 (2007).
12. D. R. Carrasco *et al.*, *Cancer Cell* **11**, 349 (2007).
13. I. Papandreou *et al.*, *Blood* **117**, 1311 (2011).
14. K. Mori, *J. Biochem.* **146**, 743 (2009).
15. A. J. Schindler, R. Schekman, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 17775 (2009).
16. K. Haze, H. Yoshida, H. Yanagi, T. Yura, K. Mori, *Mol. Biol. Cell* **10**, 3787 (1999).
17. J. Ye *et al.*, *Mol. Cell* **6**, 1355 (2000).
18. M. S. Brown, J. L. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 11041 (1999).
19. S. J. Marciniak *et al.*, *Genes Dev.* **18**, 3066 (2004).
20. P. Tsaytler, H. P. Harding, D. Ron, A. Bertolotti, *Science* **332**, 91 (2011).
21. H. P. Harding *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 1832 (2009).
22. A. M. Reimold *et al.*, *Nature* **412**, 300 (2001).
23. K. P. Lee *et al.*, *Cell* **132**, 89 (2008).
24. A. V. Korennykh *et al.*, *Nature* **457**, 687 (2009).
25. M. M. Ali *et al.*, *EMBO J.* **30**, 894 (2011).
26. A. Chawla, S. Chakrabarti, G. Ghosh, M. Niwa, *J. Cell Biol.* **193**, 41 (2011).
27. C. Rubio *et al.*, *J. Cell Biol.* **193**, 171 (2011).
28. A. V. Korennykh *et al.*, *BMC Biol.* **9**, 48 (2011).
29. R. L. Wiseman *et al.*, *Mol. Cell* **38**, 291 (2010).
30. T. Aragón *et al.*, *Nature* **457**, 736 (2009).
31. K. Yanagitani, Y. Kimata, H. Kadokura, K. Kohno, *Science* **331**, 586 (2011); 10.1126/science.1197142.
32. Y. Deng *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 7247 (2011).
33. Y. Kimata *et al.*, *J. Cell Biol.* **179**, 75 (2007).
34. H. Li, A. V. Korennykh, S. L. Behrman, P. Walter, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16113 (2010).
35. D. Pincus *et al.*, *PLoS Biol.* **8**, e1000415 (2010).
36. Y. Kimata, D. Oikawa, Y. Shimizu, Y. Ishiwata-Kimata, K. Kohno, *J. Cell Biol.* **167**, 445 (2004).
37. J. J. Credle, J. S. Finer-Moore, F. R. Papa, R. M. Stroud, P. Walter, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18773 (2005).

38. B. M. Gardner, P. Walter, *Science* **333**, 1891 (2011); 10.1126/science.1209126.
39. S. Kawaguchi, D. T. W. Ng, *Science* **333**, 1830 (2011).
40. J. Zhou *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 14343 (2006).
41. T. Promlek *et al.*, *Mol. Biol. Cell* **22**, 3520 (2011).
42. J. Maiuolo, S. Bulotta, C. Verderio, R. Benfante, N. Borge, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 7832 (2011).
43. E. van Anken *et al.*, *Immunity* **18**, 243 (2003).
44. C. C. Hu, S. K. Dougan, A. M. McGehee, J. C. Love, H. L. Ploegh, *EMBO J.* **28**, 1624 (2009).
45. J. Hollien, J. S. Weissman, *Science* **313**, 104 (2006).
46. J. Hollien *et al.*, *J. Cell Biol.* **186**, 323 (2009).
47. D. Han *et al.*, *Cell* **138**, 562 (2009).
48. J. H. Lin *et al.*, *Science* **318**, 944 (2007).
49. K. Y. Tsang *et al.*, *PLoS Biol.* **5**, e44 (2007).

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Road to Ruin: Targeting Proteins for Degradation in the Endoplasmic Reticulum

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Some nascent proteins that fold within the endoplasmic reticulum (ER) never reach their native state. Misfolded proteins are removed from the folding machinery, dislocated from the ER into the cytosol, and degraded in a series of pathways collectively referred to as ER-associated degradation (ERAD). Distinct ERAD pathways centered on different E3 ubiquitin ligases survey the range of potential substrates. We now know many of the components of the ERAD machinery and pathways used to detect substrates and target them for degradation. Much less is known about the features used to identify terminally misfolded conformations and the broader role of these pathways in regulating protein half-lives.

Proteins destined for secretion or insertion into the membrane enter the endoplasmic reticulum (ER) in an unfolded form and generally leave only after they have reached their native states. Yet, folding in the ER is often slow and inefficient, with a substantial fraction of polypeptides failing to reach the native state. Thus, the cell must continuously assess the pool of folding proteins and remove polypeptides that are terminally misfolded. This process of culling is critical to protect the cell from the toxic effects of misfolded proteins.

Remarkably, many proteins triaged as terminally misfolded are first removed from the ER via delivery (or dislocation) to the cytosol, where they are then degraded by the ubiquitin-proteasome system (*1*). This process is commonly referred to as ER-associated degradation (ERAD)—an umbrella term that covers a range of different mechanisms. Once terminally misfolded proteins are distinguished from what are likely to be structurally similar folding species, they are extracted from the pro-folding chaperone machinery, delivered to a transmembrane complex that coordinates their dislocation and, finally, escorted to the proteasome for degradation (Fig. 1A).

A convergence of genetic and biochemical studies, including work in the budding yeast *Saccharomyces cerevisiae* and in metazoan systems, has led to the identification of many of the key components involved in substrate recognition and degradation. Characterization of these components has uncovered distinct and well-conserved ERAD pathways, but for only a limited number of model substrates. We still do not know all of the endogenous targets of ERAD or the relative importance of ERAD in the quality control of misfolded conformations versus a broader role in regulating the half-lives of proteins that have reached the native state.

E3 Ubiquitin Ligases: Central Organizers

At the center of all ERAD pathways are multiprotein transmembrane complexes formed around E3 ubiquitin ligases (2–4). The E3s have variable numbers of transmembrane domains and a cytosolic RING finger domain. They catalyze substrate ubiquitylation (5) and organize the complexes that coordinate events on both sides of and within the ER membrane. When overexpressed, the yeast Hrd1p protein—the prototypical ERAD E3—can autonomously carry out degradation of soluble substrates within the ER lumen (6). This ability implicates Hrd1p—and by inference other ERAD E3s—in the physical process of transporting substrates across the ER membrane. Yet, this step remains mysterious, and it is likely that other components also facilitate dislocation.

If the E3s can act alone, then why do they form large complexes? The E3s require a dynamic complement of adaptor proteins that fa-

cilitate substrate recognition and delivery while also regulating E3 activity. In fact, overexpression of Hrd1p without its adaptors is toxic to cells, apparently because of uncontrolled and inappropriate degradation of many proteins (2). Although we understand the role of these adaptors in specific systems, such as the delivery of glycoproteins to E3s, the broader role of adaptors in restricting E3 activity to legitimate substrates remains unclear.

Individual E3s can survey overlapping but distinct ranges of substrates with diverse topologies (those with misfolded domains in the ER lumen, membrane, or cytosolic compartments) (Fig. 1B) (3). The E3s implicated in ERAD include two proteins with distinct topologies in yeast, Hrd1p (5) and Doa10p (7), and many more in metazoans, such as HRD1, gp78, RMA1 (RNF5), TRC8, and TEB4 (MARCH IV) (8). Here, we will focus on the complexes formed around the best-characterized class of E3s, the HRD ligases, which include Hrd1p in yeast as well as HRD1 and gp78 in metazoans (Fig. 1, C and D).

Adaptors control E3-substrate interactions. Adaptors are the peripheral components of the E3 complex that impart the rich substrate repertoire and stringent specificity of ERAD. Hrd3p in yeast and its metazoan counterpart SEL1L are the most thoroughly characterized adaptors (9). These proteins contain a single transmembrane domain, which in the case of Hrd3p is dispensable for function (5). Rather, the business end of the molecule is a large luminal domain composed of multiple tetratricopeptide repeats (TPRs) thought to facilitate protein-protein interactions (9). Hrd3p can bind potential substrates directly on the basis of their misfolded character (2, 9) and thus recruits misfolded proteins to the E3 ligase. Hrd3p/SEL1L also recruit other adaptors—such as the glycan-binding (lectin) protein Yos9p (9) in yeast and OS-9 and XTP3-B (10) in mammals—to the E3 complex. These lectins broaden the E3 substrate repertoire; their absence leads to a specific defect in glycoprotein degradation but does not affect degradation of other Hrd3p/SEL1L and HRD dependent substrates, such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (11).

Housekeeping chaperones may serve as adaptors. The cytoplasmic Hsp70, Ssa1p, facilitates substrate interaction with Doa10p (12); the ER-resident Hsp70s, Kar2p in yeast and BiP in mammals, interact with Yos9p-Hrd3p (2) and OS-9/XTP3-B-SEL1L (13) in a stable manner, local-

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