

Signal integration in the endoplasmic reticulum unfolded protein response

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Abstract | The endoplasmic reticulum (ER) responds to the accumulation of unfolded proteins in its lumen (ER stress) by activating intracellular signal transduction pathways — cumulatively called the unfolded protein response (UPR). Together, at least three mechanistically distinct arms of the UPR regulate the expression of numerous genes that function within the secretory pathway but also affect broad aspects of cell fate and the metabolism of proteins, amino acids and lipids. The arms of the UPR are integrated to provide a response that remodels the secretory apparatus and aligns cellular physiology to the demands imposed by ER stress.

ER stress

The consequence of a mismatch between the load of unfolded and misfolded proteins in the endoplasmic reticulum and the capacity of the cellular machinery that copes with that load.

In eukaryotic cells, most secreted and transmembrane proteins fold and mature in the lumen of the endoplasmic reticulum (ER). Proteins enter the ER as unfolded polypeptide chains. Their flux into the ER is variable because it can change rapidly in response to programmes of cell differentiation, environmental conditions and the physiological state of the cell. To handle this dynamic situation, cells adjust the protein-folding capacity of the ER according to their requirements, thereby ensuring that the quality of cell-surface and secreted proteins can be maintained with high fidelity. Such homeostatic control is achieved through the action of signal transduction pathways that have sensors facing the ER lumen and effectors that convey the message to other compartments of the cell. The first clue to the existence of such intracellular signalling events was provided by the observation that pharmacological and genetic manipulations that increase the load of unfolded ER proteins selectively activate the expression of genes that encode ER-resident chaperones¹. The intracellular signalling pathway that mediates this regulation was named the unfolded protein response (UPR).

The principles of the UPR are now relatively well defined. An imbalance (called ER stress) between the load of unfolded proteins that enter the ER and the capacity of the cellular machinery that handles this load sets three main responses in motion, the first two of which are rectifying. First, there is a reduction in the protein load that enters the ER, which is a transient adaptation that is achieved by lowering protein synthesis and translocation into the ER. Second, there is an increase in the capacity of the ER to handle unfolded proteins, which is a longer-term adaptation that entails transcriptional activation of

UPR target genes, including those that function as part of the ER protein-folding machinery. If homeostasis cannot be re-established then a third mechanism, cell death, is triggered, presumably to protect the organism from rogue cells that display misfolded proteins.

Three different classes of ER stress transducers have been identified. Each class defines a distinct arm of the UPR that is mediated by inositol-requiring protein-1 (IRE1), activating transcription factor-6 (ATF6) or protein kinase RNA (PKR)-like ER kinase (PERK). In each case, an integral membrane protein senses the protein-folding status in the ER lumen and transmits this information across the ER membrane to the cytosol. The stress transducers and their downstream effectors have been the subject of detailed reviews^{2,3}. Less has been written on how signalling in the UPR is integrated to remodel the secretory pathway and how the UPR relates to cell survival during ER stress; hence, this Review summarizes recent developments in these areas.

IRE1: the conserved core of the UPR

Signalling in the UPR is initiated by ER transmembrane proteins, which have luminal portions that sense the protein-folding environment in the ER, and cytoplasmic effector portions that interact with the transcriptional or translational apparatus. The first such stress transducer was identified by a screen for mutations that block the activation of a UPR-inducible reporter in yeast. The gene in question, *IRE1*, encodes a type I ER-resident transmembrane protein with a novel luminal domain and a cytoplasmic portion that contains a protein kinase domain^{4,5} (FIG. 1). In response to unfolded proteins, IRE1 oligomerizes in the plane of the membrane, allowing

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doi:10.1038/nrm2199

Published online 13 June 2007

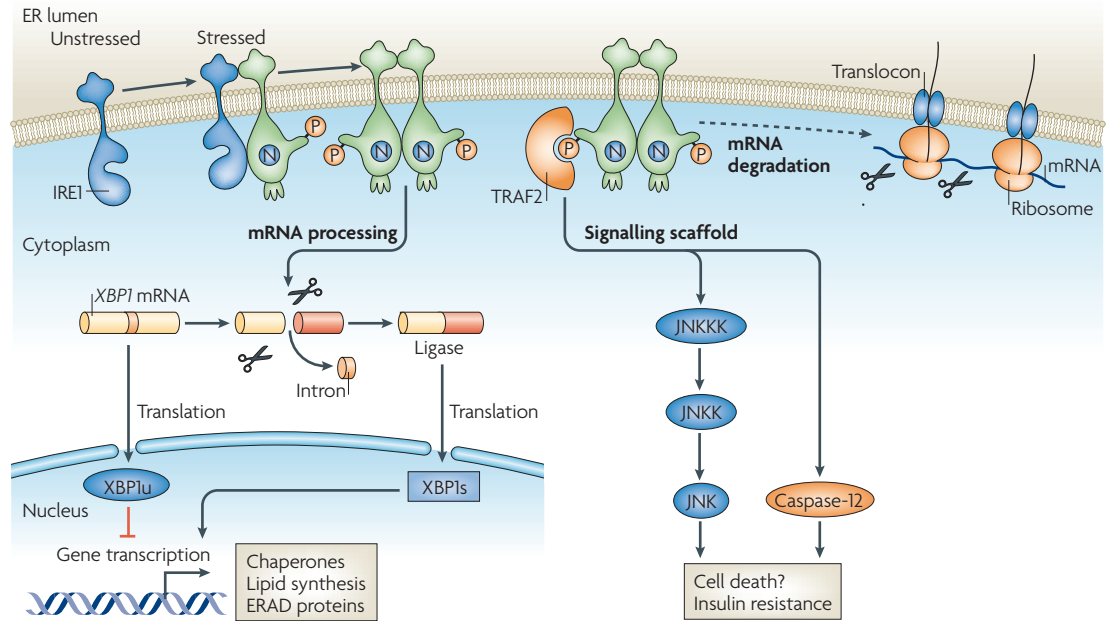


Figure 1 | Signalling by IRE1. Inositol-requiring protein-1 (IRE1) oligomerizes in the plane of the endoplasmic reticulum (ER) membrane in stressed cells. *Trans*-autophosphorylation in its cytosolic kinase domain increases the affinity for nucleotides (N), which allosterically activate IRE1 (REF. 9) and unmask a dormant endoribonucleolytic activity⁹⁷. IRE1-mediated sequence-specific cleavage of a single known mRNA (*X*-box binding protein-1 (*XBP1*) in higher eukaryotes, *HAC1* (homologous to *ATF/CREB1*) in yeast) excises a small RNA fragment (intron). The two ends of the mRNA are ligated (tRNA ligase (*Trl1*) has this role in yeast¹⁴ but the identity of the ligase is unknown in metazoans), which leads to a frame shift in the coding sequence (shown in the figure as a colour change from yellow to red after removal of the intron). Spliced *XBP1* mRNA encodes a potent transcriptional activator (*XBP1s*), whereas the unspliced *XBP1* mRNA encodes *XBP1u*, an inhibitor of the unfolded protein response (UPR)¹⁷. In yeast, the *Hac1/XBP1* pathway activates most of the UPR, whereas in mammals, it appears that *XBP1* regulates a subset of UPR genes that promote ER-associated degradation (ERAD) of misfolded proteins and ER biogenesis⁴⁸. IRE1 can also act by alternative means. In mammals, recruitment of TRAF2 (tumour necrosis factor receptor (TNFR)-associated factor-2) by phosphorylated IRE1 allows it to signal to Jun N-terminal kinase (JNK)²² and alter intracellular signalling (for example, resulting in insulin resistance⁶⁶). The IRE1–TRAF2 complex has also been linked to caspase-12 activation and cell death²³. In cultured *Drosophila melanogaster* cells, activated IRE1 can promote the cleavage of various ER-localized mRNAs, leading to their degradation²⁹. This reduces the load on the stressed ER and might facilitate reprogramming of the ER-associated protein synthesis and translocation machinery. It is unknown whether IRE1 cleaves these mRNAs directly or whether it promotes their degradation by activating or recruiting other RNases. JNKK, JNK kinase; JNKKK, JNKK kinase.

for *trans*-autophosphorylation of juxtaposed kinase domains. Oligomerization can be triggered directly by binding of unfolded proteins to the IRE1 luminal domain (which bears an architectural resemblance to the peptide-binding domains of major histocompatibility complexes^{6,7}), or might involve the release of oligomerization-repressing chaperones, or both (BOX 1). Contrary to expectations, however, IRE1 signalling does not entail a conventional cascade of sequential kinase activation because the only known substrate of the IRE1 kinase is IRE1 itself^{8,9}. By a poorly understood mechanism, *trans*-autophosphorylation of the kinase domain of IRE1 activates its unusual effector function, which causes the precise endonucleolytic cleavage of the only known substrate: an mRNA that encodes a transcription factor named *Hac1* (homologous to *ATF/CREB1*) in yeast^{10,11} or *XBP1* (*X*-box binding protein-1) in metazoans^{12,13} (FIG. 1). IRE1 is therefore a bifunctional enzyme, possessing both a protein kinase and a site-specific endoribonuclease that is regulated by its intrinsic kinase module.

IRE1 cuts the precursor *Hac1* or *XBP1* mRNA twice, excising an intervening fragment or intron. The 5' and 3' mRNA fragments are then ligated, generating a spliced mRNA that encodes an activator of UPR target genes. Biochemical and genetic evidence indicates that in yeast, ligation of the two ends of the *HAC1* mRNA is mediated by tRNA ligase (*Trl1*)¹⁴. The enzyme(s) responsible for this reaction in higher eukaryotes have not been identified, but recent evidence for a tight association of *XBP1* mRNA with membranes¹⁵ indicates that, as in yeast, cleavage and ligation occur in association with the ER.

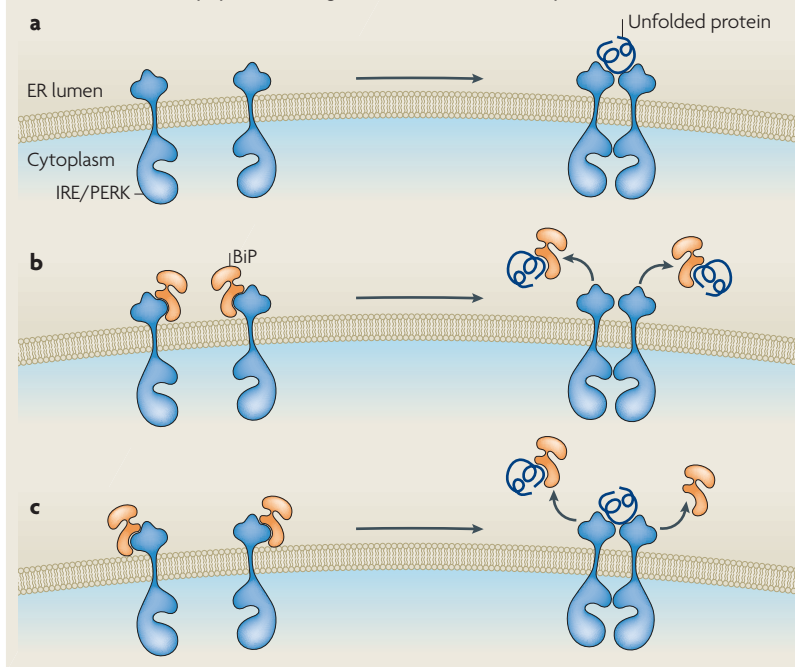
The consequences of this IRE1-dependent splicing event differ in yeast and metazoans. In yeast, the *HAC1* mRNA intron represses translation and relief of this repression is the key activating event of the yeast UPR¹⁶. By contrast, in metazoans both the precursor and spliced form of *XBP1* are translated^{13,17}. However, the encoded proteins, which differ markedly in sequence owing to a splicing-mediated frame shift, have different functional properties. The form of *XBP1* encoded in the spliced mRNA is more stable¹³ and works as a potent activator of UPR

Major histocompatibility complex

A plasma-membrane protein complex that binds and displays immunogenic peptides in a characteristic groove structure.

Box 1 | How is ER stress sensed?

The endoplasmic reticulum (ER) stress transducers inositol-requiring protein-1 (IRE1) and protein kinase RNA (PKR)-like ER kinase (PERK) are enzymes whose oligomerization-induced activation is linked to perturbed protein folding in the ER. Both IRE1 and PERK contain experimentally interchangeable and evolutionarily related unfolded protein-sensing domains that are located in the ER lumen. Three models for how these domains sense the unfolded protein load have been proposed. The direct recognition model proposes that unfolded proteins bind directly to the luminal domains of IRE1 and PERK (panel a). The recent crystal structure of the yeast Ire1 luminal domain revealed that an Ire1 dimer forms a deep, long groove, with dimensions that are consistent with the ability to bind an extended polypeptide⁹. Access to the groove was obstructed in the otherwise similar crystal structure of mammalian IRE1 α ⁷, but it is unclear how these differences reflect on the function of IRE1. The possibility of peptide binding by IRE1 (and PERK, the luminal domain of which can easily fit into a homologous groove-containing dimeric structure) suggests that peptide binding drives oligomerization. An alternative, indirect recognition model posits that binding of the abundant ER chaperone immunoglobulin-binding protein (BiP) locks IRE1 and PERK into the inactive state (panel b). Indeed, in the unstressed state, both proteins are in a complex that contains BiP. ER stress correlates with dissociation of the inhibited complex and the incorporation of PERK or IRE1 into higher order, active complexes. Furthermore, BiP overexpression markedly attenuates PERK and IRE1 activity and represses the unfolded protein response (UPR)^{31,85}, whereas reducing BiP levels activates the UPR. The model in panel b, in which binding of BiP to the luminal domains of IRE1 and PERK represses activation, is consistent with these observations. The indirect recognition model, however, does not readily explain how the high molar ratio of BiP to stress transducers can be reconciled with the sensitivity of the UPR to subtle changes in levels of BiP clients (the unfolded proteins). Also, BiP binding is not essential to IRE1 regulation^{6,86}. A third, hybrid recognition model proposes that both BiP dissociation and peptide binding cause sensor activation (panel c).



target genes¹², whereas the protein encoded by the precursor mRNA is labile and represses UPR target genes.

In addition to post-transcriptional regulation by IRE1-dependent splicing, *HAC1* and *XBPI* mRNAs are also transcriptional targets of the UPR. In yeast, *HAC1* mRNA production is induced under conditions of severe protein misfolding in the ER, and the resulting increase in the Hac1 transcription factor drives a qualitatively different transcription programme called the super-UPR¹⁸. In metazoan cells, levels of *XBPI*

mRNA also increase upon UPR induction and continue to rise as ER stress declines and IRE1 is inactivated¹⁷. Therefore, the new *XBPI* mRNA remains in its precursor, unspliced form, which encodes an inhibitor of XBP1 signalling. This might serve to terminate signalling by a combination of inhibitory heterodimerization with spliced XBP1 and competition for binding sites¹⁷. The XBP1 form derived from the unspliced mRNA might also sharpen the response, conferring a switch-like property to XBP1-mediated gene regulation.

Yeast *IRE1* and *HAC1* function in a linear pathway, as revealed by profiling, which showed that there was substantial overlap of the defect in gene expression in cells that lacked either gene¹⁹. The situation in higher eukaryotes is less clear. Mouse embryos that lack either IRE1 or XBP1 perish at a similar early stage of gestation, suggesting that XBP1 mediates critical functions of IRE1. Furthermore, genome-wide computational approaches have uncovered no other mammalian mRNAs with overlapping long open reading frames and a short intron flanked by IRE1 cleavage sites (features that are expected of IRE1 substrates)²⁰. However, expression profiling in *Caenorhabditis elegans* has revealed non-overlapping defects in animals with mutations in *ire-1* and *xbp-1*, which suggests the existence of alternative IRE-1 effectors in that species²¹. Metazoan IRE1 probably has signalling functions beyond its nucleolytic activity. For example, mammalian IRE1 activates the stress-induced Jun N-terminal kinase (JNK)²² and interacts with components of the cell-death machinery, such as caspase-12, independently of its RNase activity^{23,24}. However, apart from indirect evidence for a contribution to the death of ER-stressed cells²⁵, the physiological significance of such pathway branches remains unclear.

Recently, analysis of mRNAs expressed in ER-stressed *Drosophila melanogaster* cells uncovered evidence for widespread IRE1-dependent degradation of ER-associated mRNAs²⁶. This process is likely to represent an XBP1-independent post-transcriptional mechanism for IRE1 control of gene expression that remodels the repertoire of proteins translated in ER-stressed cells. It is unknown whether the nuclease that initiates these events is IRE1 itself, functioning in a mode of much relaxed specificity, or another, yet-to-be-identified nuclease that is recruited or locally activated by IRE1.

ATF6: the UPR and regulated proteolysis

A search for additional proteins that bind UPR-activated promoter elements led to the identification of ATF6, a founding member of a novel class of metazoan-specific ER stress transducers²⁷. These are synthesized as inactive precursors, tethered to the ER membrane by a transmembrane segment and have a stress-sensing portion that projects into the ER lumen. Under conditions of ER stress, ATF6 is transported from the ER to the Golgi apparatus, where it is cleaved by Golgi-resident proteases, first by S1P (site 1 protease) and then in an intra-membrane region by S2P (site 2 protease) to release the cytosolic DNA-binding portion, ATF6f ('f' for fragment). From there, ATF6f moves to the nucleus to activate gene expression²⁷ (FIG. 2).

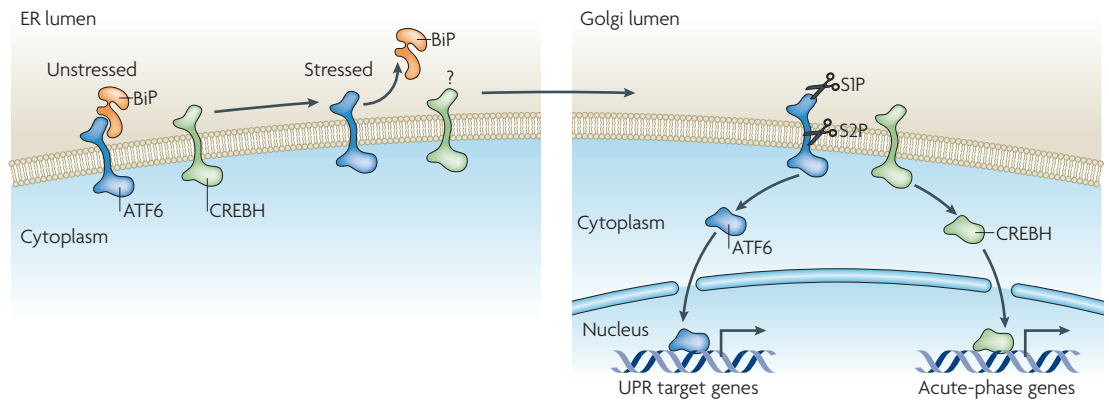


Figure 2 | Signalling by ATF6. Activating transcription factor-6 (ATF6) and cyclic AMP response element binding protein hepatocyte (CREBH) are transmembrane proteins with a cytoplasmic portion that, when liberated from its transmembrane tether, can bind to DNA and activate target genes^{27,30}. In unstressed cells, ATF6 and CREBH reside in the endoplasmic reticulum (ER) membrane. ATF6 trafficking appears to be hindered by binding of the ER chaperone immunoglobulin-binding protein (BiP) to its luminal domain. ER stress disrupts BiP binding and ATF6 (and CREBH) are delivered to the Golgi apparatus²⁹. The details of this vesicular transport event remain unknown. In the Golgi apparatus, these proteins are subject to consecutive cleavage, first by the luminal site 1 protease (S1P) and then the intra-membrane site 2 protease (S2P), which liberates the cytosolic effector portions of the proteins from the membrane and allows their import into the nucleus²⁸. ATF6 probably activates a subset of UPR target genes, although these remain to be characterized, whereas CREBH activates acute-phase response genes that encode secreted proteins involved in inflammation³⁰.

The proteolytic machinery for regulated intra-membrane proteolysis of ATF6 proteins is shared with the sterol response element binding proteins (SREBPs)²⁸. In both cases, trafficking of the inactive precursor to the Golgi apparatus precedes the activating proteolytic steps. However, the initiating signal for the trafficking event is different for the two classes of proteins: release from sterol repression in the case of SREBPs, but an increase of unfolded proteins in the ER in the case of ATF6. This increase might involve direct or indirect (chaperone-mediated²⁹) sensing of unfolded proteins by the luminal domain of ATF6 proteins, as discussed above for IRE1 (BOX 1), which results in the trafficking of ATF6 from the ER to the Golgi.

The predicted structures of several other proteins suggest that they are ER-anchored transcription factors that are related to ATF6 (for example, LZIP (also known as human or cyclic AMP-responsive element binding protein-3 (CREB3), OASIS (also known as CREB3-like-1) and Tisp40 (transcript induced in spermiogenesis-40)). One such protein, CREB-hepatocyte (CREBH), has recently been found to be activated by ER-stress-regulated proteolysis. However, CREBH does not activate genes that enhance the capacity of the secretory pathway but, rather, links ER stress in the liver to the secretion of serum proteins that are associated with inflammation (so-called acute-phase responsive proteins)³⁰. These observations are an intriguing example of integration of the UPR with a range of physiological systems.

PERK and translational control

The third ER stress transducer, PERK, superficially resembles IRE1. Both are ER-localized type I transmembrane proteins with luminal stress-sensing domains that are phylogenetically related, similar in structure

and function, and experimentally interchangeable³¹. The cytoplasmic portion of PERK also contains a protein kinase domain, which undergoes activating *trans*-autophosphorylation by oligomerization in ER-stressed cells; however, unlike IRE1, for which the only substrate is itself, PERK phosphorylates the α -subunit of eukaryotic translation initiation factor-2 (eIF2 α) at Ser51. This phosphorylation inhibits the guanine nucleotide exchange factor eIF2B, a pentameric complex that recycles eIF2 to its active GTP-bound form. Lower levels of active eIF2 result in lower levels of translation initiation, globally reducing the load of newly synthesized proteins, many of which are destined to enter the already stressed ER lumen³² (FIG. 3).

In addition to decreasing global protein synthesis to reduce the ER load, PERK-mediated eIF2 α phosphorylation also contributes to transcriptional activation in the UPR. Expression profiling in ER-stressed PERK knockout cells showed the defective induction of numerous mRNAs that are responsible for the normal UPR³³. A similar defect in stress-induced gene expression was also observed in cells with a Ser51Ala mutation in eIF2 α that prevents the regulatory phosphorylation event but does not otherwise affect eIF2 function³⁴. Furthermore, most genes that are downregulated in PERK-knockout cells were induced by the directed activation of PERK (in an experimental system that is uncoupled from ER stress). Most importantly, the entire range of the PERK-dependent gene expression programme required eIF2 α phosphorylation as it was blocked by the Ser51Ala mutation³⁵.

The link between eIF2 α phosphorylation and activated gene expression is conserved in eukaryotes. In yeast, phosphorylation of eIF2 α by Gcn2 (general control non-repressible-2; a kinase activated by uncharged transfer RNAs) leads to translational upregulation of the

Sterol response element binding protein

A protein from a family of ER-localized membrane-anchored transcription factors that are activated by sterol depletion to bind genes involved in sterol and fatty acid synthesis.

Acute-phase responsive protein

A serum protein secreted by the liver and, to a lesser degree, by other cells in response to systemic inflammation.

Uncharged transfer RNA

A transfer RNA that has not been amino-acylated by its (cognate) amino acid.

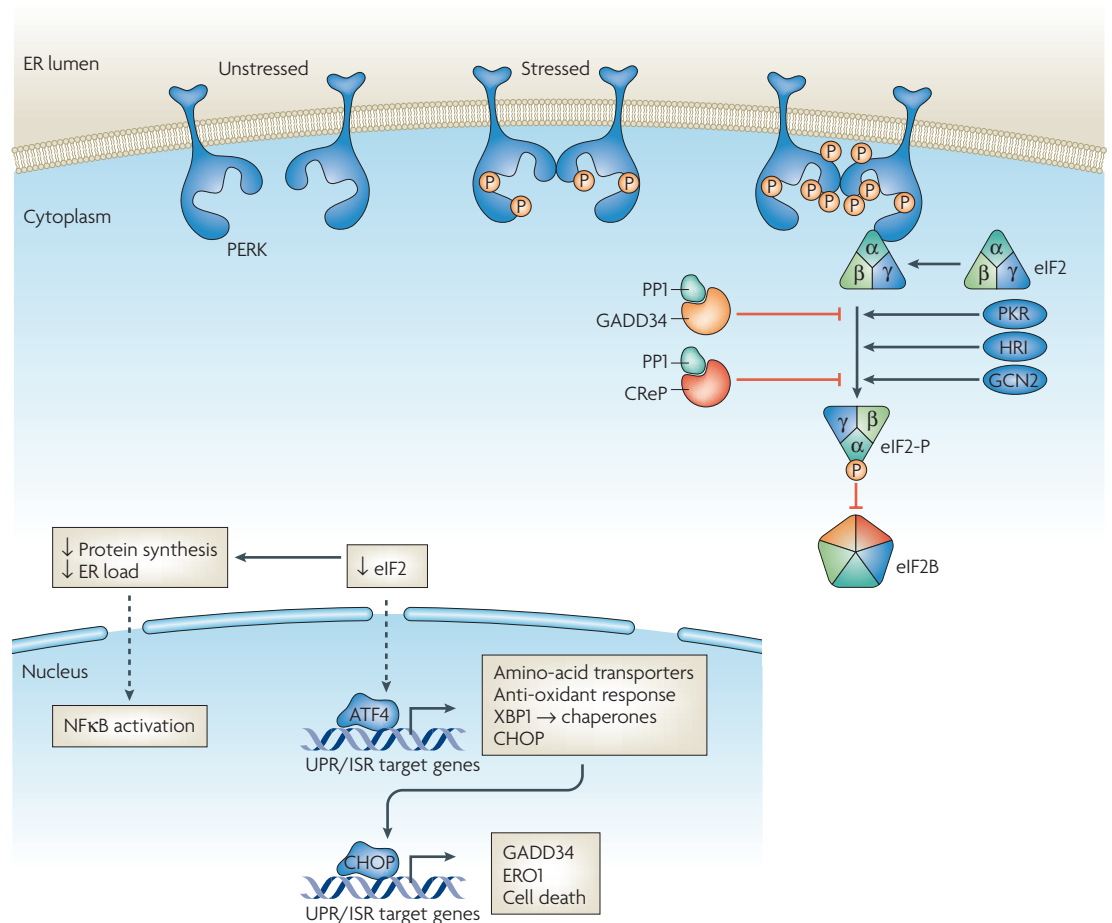


Figure 3 | Signalling by PERK to the translational machinery. In response to endoplasmic reticulum (ER) stress, protein kinase RNA (PKR)-like ER kinase (PERK), similar to inositol-requiring protein-1 (IRE1), oligomerizes in the plane of the membrane and is activated by *trans*-autophosphorylation of its activation loop³¹. Extensive further phosphorylation of the large kinase insert loop facilitates substrate recruitment⁹⁸. Phosphorylation of a single known substrate, the α subunit of eukaryotic translation initiation factor-2 (eIF2) on Ser51, inhibits the pentameric guanine nucleotide exchange factor eIF2B from recycling eIF2 to its active GTP-bound form. The resulting reduced activities of eIF2B and the eIF2 complex account for all of the important consequences of PERK activity. Because other eIF2 kinases (PKR, haem-regulated inhibitor kinase (HRI) and general control non-repressible-2 (GCN2)) can activate this pathway independently of ER stress, this portion of the unfolded protein response (UPR) is termed the integrated stress response (ISR)^{33,52}. Lower global protein synthesis reduces ER unfolded protein load but also affects gene transcription. For example, translation of the activating transcription factor-4 (ATF4) is increased under conditions of limiting eIF2, whereas nuclear factor κ B (NF κ B) is activated post-translationally. The ISR activates genes that encode amino-acid transporters and genes that protect against oxidative stress, and it contributes to the transcriptional activation of XBP1 (REF. 52). The transcription factor CHOP (C/EBP-homologous protein) is also activated transcriptionally by ATF4 and its target genes include GADD34 (growth arrest and DNA damage-inducible protein-34), a regulatory subunit of phosphatase PP1 that dephosphorylates eIF2 α and terminates signalling in the ISR⁷⁷, and ER oxidase-1 (ERO1), which is required for disulphide bond formation in protein folding. A constitutive phosphatase CReP (constitutive repressor of eIF2 α phosphorylation) assists GADD34 in this task⁴¹.

transcription factor Gcn4, which is a key regulator of the cellular response to amino-acid deprivation³⁶. The 5'-untranslated region of *GCN4* contains short, inhibitory upstream open reading frames (uORFs) that prevent translation of the downstream *GCN4*-encoding ORF in unstressed cells. Conditions that limit eIF2 activity (due to eIF2 α phosphorylation) lead to ribosomes skipping the inhibitory uORFs so that the *GCN4* ORF can be translated. This feature is conserved in mammalian ATF4 (the metazoan homologue of Gcn4), a transcription factor that is translationally induced by phosphorylation of eIF2 α (REFS 37,38).

At high levels, eIF2 α phosphorylation can also activate nuclear factor κ B (NF κ B), but there is controversy about the mechanism(s) involved. In one study, conditions that were associated with eIF2 α phosphorylation led to the physical disruption of an inhibitory complex that contains NF κ B (REF. 39), whereas other work indicates that the translational repression that is mediated by eIF2 α phosphorylation causes decreased levels of the repressor of NF κ B (REF. 40). Regardless of mechanism, the contribution of NF κ B to ATF4-independent, PERK-dependent UPR target gene expression remains to be defined, and other effectors of PERK signalling to the genome by

eIF2 α phosphorylation need to be identified. Importantly, several other signalling pathways unrelated to ER stress (triggered by amino-acid starvation, double-stranded RNA accumulation or haem depletion) also converge on eIF2 α phosphorylation and activate a common set of target genes. Because of this integrative feature, signalling downstream of phosphorylated eIF2 α was termed the integrated stress response (ISR)³³.

There is little doubt that cells must tightly regulate the level of phosphorylated eIF2 α to survive. PERK activation by ER stress is rapidly reversible and, within minutes of restoring ER homeostasis, activated PERK is dephosphorylated^{31,41}. The regulatory mechanisms and the phosphatase(s) involved remain unknown, but it has been established that phosphorylated eIF2 α is also subject to negative regulation. Two components of this process have been identified by somatic-cell genetic screens for genes that, when overexpressed, block the activation of a PERK-dependent reporter. The two identified genes, *GADD34* (growth arrest and DNA-damage-inducible protein-34) and *CREP* (constitutive repressor of eIF2 α phosphorylation), encode the substrate targeting subunits of two phosphatase complexes that independently dephosphorylate eIF2 α (REFS 41–43). CREP is constitutively expressed and contributes to baseline eIF2 α dephosphorylation⁴¹, whereas GADD34 is induced as part of the gene expression programme activated by eIF2 α phosphorylation and serves in a negative feedback loop that operates within it^{44–46}.

Cross-talk between the arms of the UPR

Whereas IRE1, PERK and ATF6 activation proceeds independently in ER-stressed cells, the three arms of the UPR communicate with each other extensively. Little is known about the role of ATF6 family members in regulating UPR-mediated gene expression and the phenotypes of loss-of-function mutations in mammalian *ATF6* genes have not been described. However, experiments in *C. elegans* indicate that there is functional redundancy between the IRE1–XBP1 arm and the ATF6 arm of the UPR. This is inferred from the observation that mutations in either arm are relatively well tolerated, but compromising both arms blocks worm development²¹. It is unclear, however, if this genetic complementation reflects the redundant activation of a common set of genes or functional redundancy between distinct sets of ATF6 and XBP1 target genes.

PERK and eIF2 α phosphorylation have an important role in protecting cells against the consequences of ER stress^{34,47}. However, it is currently impossible to separate the effects of eIF2 α phosphorylation on protein synthesis from its effects on activated gene expression; thus, we are unable to gauge the relative contribution of the two components to homeostasis during ER stress. Furthermore, the transcriptional effects of the three known arms of the UPR overlap significantly, which is achieved in part through mutual positive reinforcement. For example, XBP1 is transcriptionally activated by ATF6 (REF. 12) and PERK signalling¹³, the latter of which might account for the broad defect in the UPR in cells that lack PERK. Nonetheless, there appear to be important differences in the direct targets of the transcriptional activators of the UPR⁴⁸.

Yeast Gcn4, similar to its metazoan homologue ATF4, also collaborates with Hac1 in the UPR⁴⁹ and is the main mediator of eIF2 α -phosphorylation-dependent gene expression in yeast. By contrast, expression profiling of mammalian cells shows that approximately half of the PERK-dependent UPR target genes are ATF4 independent³³, which points to the existence of other PERK effectors downstream of phosphorylated eIF2 α . Attempts to identify other effectors have so far been unsuccessful.

Remodelling of the ER by stress

The size of the ER correlates with the unfolded protein load across different cell types and different physiological states. Several lines of evidence suggest that the UPR contributes to the coupling of ER expansion to physiological demand. The first hint was the surprising finding that sensors of the UPR respond to an imbalance between unfolded proteins and chaperones in the ER lumen, yet the UPR also activates processes such as amino acid import and tRNA charging that cannot be understood merely in terms of restoring the buffering of unfolded proteins by chaperones^{50,51}. Amino-acid transporters, for example, are encoded by UPR target genes that are activated by PERK-mediated eIF2 α phosphorylation. Their activation might further threaten the balance of chaperones and unfolded proteins because they promote sustained protein synthesis, which loads the ER with unfolded proteins. These findings indicate that the UPR broadly functions to increase the capacity of a cell to carry out protein secretion rather than narrowly defending cells against ER stress. It is therefore likely that the UPR protects cells from ER stress and increases secretory capacity by carefully attending to the sequence in which various mechanisms that remodel the ER are deployed.

Reprogramming translation and translocation. A reduction in the global rates of translation initiation due to PERK-mediated eIF2 α phosphorylation is one of the earliest events in ER stress. In addition to reducing the load on the ER, eIF2 α phosphorylation liberates ribosomes and translation factors from mRNA, and the latter accumulate as free subunits⁵². This resetting of the translational programme is predicted to help newly synthesized mRNAs that are transcribed by the UPR-induced gene-expression programme to compete for limiting translation factors.

The reprogramming of ER translation is also proposed to be mediated, in part, by a recently identified process whereby mRNAs that encode secreted proteins are selectively degraded in ER-stressed cells²⁶. This phenomenon was discovered by comparing the profiles of mRNAs expressed in wild-type, IRE1-knockdown or XBP1-knockdown cells that were either unstressed or ER stressed. Numerous mRNAs that encode secreted proteins were degraded in both wild-type and XBP1-knockdown ER-stressed cells, but not in cells lacking IRE1. Further experiments showed that degradation occurs in a subset of mRNAs that must be physically associated with the ER membranes. This selective degradation of mRNAs that encode secreted proteins is predicted to reduce ER load and liberate ribosomes and translation factors that serve in reprogramming the repertoire of the ER.

Integrated stress response

The consequences of eIF2 α phosphorylation on Ser51, which is effected by four different kinases that respond to diverse upstream stress signals, including ER stress.

Amino-acid transporter

A plasma-membrane protein that transports amino acids into cells. Many are transcriptionally activated by ATF4 and, thus, are dependent on the activity of PERK.

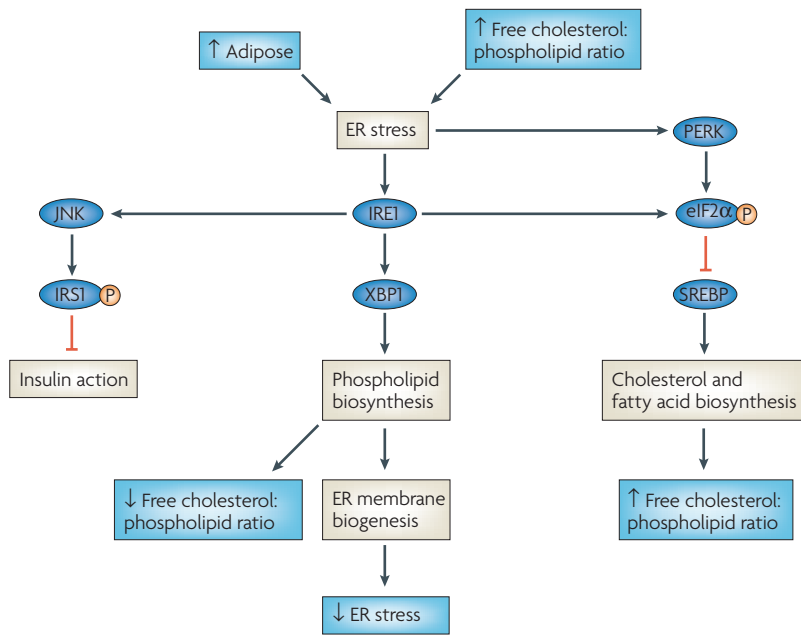


Figure 4 | ER stress and lipid metabolism. Cholesterol accumulation in endoplasmic reticulum (ER) membranes can cause ER stress, as can obesity, a metabolic state that is associated with the accumulation of cellular lipids. ER stress activates inositol-requiring protein-1 (IRE1), which leads to X-box binding protein-1 (XBP1)-dependent enhancement of phospholipid biosynthesis⁵⁹, which in turn increases membrane biogenesis. This feature of the unfolded protein response is conserved in all eukaryotes. XBP1 activity thereby reduces the free cholesterol to phospholipid ratio — a high ratio can compromise ER function⁶² and induce ER stress⁶³. Protein kinase RNA (PKR)-like ER kinase (PERK)-mediated phosphorylation of eukaryotic translation initiation factor-2 α (eIF2 α) antagonizes sterol-regulated enhancer binding protein (SREBP) activation to decrease cholesterol synthesis, although the mechanisms remain incompletely understood⁶⁴. Increased adipose stores can also promote ER stress in certain key insulin-responsive tissues, such as fat and the livers of obese mice. The consequent enhanced activity of IRE1 is linked to insulin resistance through activation of the Jun N-terminal kinase (JNK), which phosphorylates insulin receptor substrate-1 (IRS1)⁶⁶ to downregulate insulin signalling. Finally, eIF2 α phosphorylation inhibits obesity in mice by unknown mechanisms⁹⁹.

Proteins with weaker signal peptides, such as the prion precursor protein, are preferentially excluded from the ER⁵⁴. These findings led Hegde and colleagues to suggest that signal peptide strength is under negative selection to ensure that only selected proteins (for example, the ER chaperone immunoglobulin-binding protein (BiP)) are translocated into the ER under stressful conditions. This process would protect the ER and the organism by reducing the load of misfolding-prone proteins that enter the stressed organelle and, by clearing the way for newly-synthesized UPR target proteins, reprogramme the ER to meet changed needs.

Lipids and the UPR. Transcriptional and translational reprogramming enhances the synthesis of proteins that function in the ER and elsewhere in the secretory pathway. However, the UPR also contributes to an expansion of the lipid component of membranes in cells that are burdened with a heavy secretory load. In yeast, many of the genes that encode the key rate-limiting enzymes in lipid biogenesis are upregulated following UPR induction⁵⁰. IRE1 mutant yeast are dependent on exogenous sources of inositol to survive, which reflects a role for the UPR in maintaining the biosynthesis of this essential building block of phospholipids⁵⁵. Furthermore, the roles of IRE1 and XBP1 in endomembrane proliferation and phospholipid biosynthesis are conserved in mammals. This probably explains the essential role of XBP1 in the development of several types of secretory cells^{56,57}, and the observation that deregulated expression of XBP1 can single-handedly promote phospholipid biosynthesis and membrane expansion in mammalian cells^{58,59} (FIG. 4). Similarly, in yeast cells, expression of the spliced version of *HAC1* mRNA drives expansion of the ER⁶⁰. It remains unclear, however, if signalling in the UPR is sufficient to drive the remarkable expansion of the ER that is observed in professional secretory cells.

Phospholipid depletion activates the UPR^{55,61}. It is not known whether this reflects an indirect perturbation of the protein-folding environment in the ER lumen or whether IRE1 (or PERK or ATF6) can directly sense changes in the lipid composition of the ER bilayer. Interestingly, the SREBPs, which regulate the main pathway for sensing lipid sufficiency in animal cells, are also controlled through an ER connection and share components of their activating machinery with the UPR²⁸. However, there is little evidence for shared targets or for overlap between the circumstances under which the two pathways are activated. For example, deregulated XBP1 does not increase cholesterol stores⁵⁹, which is consistent with the idea that the UPR is designed to increase the production of sterol-poor ER membranes.

The UPR and the sterol-activated signalling pathways might oppose each other: cholesterol accumulation in ER membranes promotes ER stress⁶² and activates the UPR⁶³, whereas PERK-mediated eIF2 α phosphorylation interferes with SREBP activation⁶⁴ and ATF6 has been reported to antagonize SREBP2 (REF. 65). The functional significance of these relationships has not been explored in detail. It has also been reported that obesity increases the levels of ER stress in adipocytes and liver cells by an

The induction of chaperone-encoding genes by the IRE1, ATF6 and PERK pathways seems to work at cross-purposes with the repression of mRNA translation. However, GADD34-mediated eIF2 α dephosphorylation, which is deployed as part of the UPR gene-expression programme, coordinates the recovery of eIF2 activity (and translation initiation) with the transcriptional induction of UPR target genes, promoting the translation of their mRNAs^{43,46}. It is predicted that this chain of events transiently reprogrammes the ER away from its normal load of secretory proteins to the synthesis of UPR targets, which enhance the capacity of the entire secretory apparatus to handle proteins.

A conceptually similar and complementary process might also occur at the translocon, through which nascent polypeptides enter the ER. Translocation of nascent chains into the ER lumen can proceed without participation of luminal chaperones, but there is evidence that chaperones can help⁵³. ER stress, which challenges the chaperone reserve, might therefore render translocation less efficient. However, this impediment to translocation affects different proteins to different degrees.

Translocon

A proteinaceous channel in the ER membrane, through which nascent proteins are translocated into the ER lumen.

Chaperone reserve

The capacity of an organelle to tolerate a further load of unfolded proteins.

Signal peptide

The portion of a secreted protein that specifies translocation into the ER. It is usually cleaved off following translocation.

Obesity

A metabolic state characterized by excessive accumulation of triglycerides in adipose tissue.

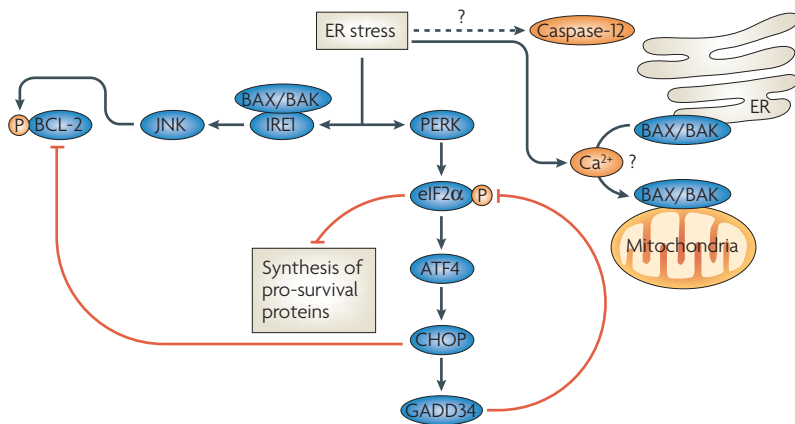


Figure 5 | ER stress and cell death. Cell death by endoplasmic reticulum (ER) stress occurs by a poorly understood mechanism. Altered calcium handling might be implicated in the translocation of the death effectors BAX and BAK from the ER to the mitochondria¹⁰⁰ and, in mice, caspase-12 activation (perhaps through tumour necrosis factor receptor (TNFR)-associated factor-2 (TRAF2)²³ is implicated in cell death⁷¹. Inositol-requiring protein-1 (IRE1)-mediated activation of Jun N-terminal kinase (JNK) might contribute to cell death by phosphorylating and inactivating the anti-apoptotic regulator BCL-2. The formation of a complex with the pro-death proteins BAX and BAK might assist in IRE1 activation²⁴. Protein kinase RNA (PKR)-like ER kinase (PERK)-mediated phosphorylation of eukaryotic translation initiation factor-2 α (eIF2 α) can contribute to cell death by inhibiting the synthesis of pro-survival proteins; one of the downstream targets of PERK, the transcription factor CHOP, may repress BCL-2 expression⁷⁴. In most circumstances, however, PERK signalling is protective against cell death and the survival benefit of CHOP knockout is best explained in terms of its role in a negative feedback loop that operates in the unfolded protein response to promote eIF2 α dephosphorylation and recovery of protein synthesis. ATF4, activating transcription factor-4; GADD34, growth arrest and DNA-damage-inducible protein-34.

unknown mechanism⁶⁶. The physiological consequences are interesting because in mammalian cells IRE1 activates JNK, which in turn suppresses signalling downstream of the insulin receptor. The potential physiological significance of this molecular mechanism is supported by the observation that insulin signalling in obese mice is markedly improved by small molecules that promote protein folding (chemical chaperones) and, thus, ameliorate ER stress⁶⁷. These provocative studies implicate ER stress in the development of obesity-linked insulin resistance and **type II diabetes**.

Removal of misfolded proteins and damaged ER. Targets of the UPR include key genes that are involved in ER-associated protein degradation (ERAD)⁵⁰. ERAD mediates the retro-translocation of unfolded proteins from the ER lumen into the cytosol for degradation by the proteasome. Therefore, ERAD complements other UPR targets — such as chaperones and protein-modifying enzymes (the upregulation of which facilitates protein folding) — by removing misfolded proteins from the ER. Proteins that enter the ERAD pathway, however, must traverse the membrane in reverse and, presumably, do so as an unfolded chain through a protein translocation channel in the membrane.

Severely misfolded proteins and protein aggregates might be difficult to unravel and degrade by this mechanism. However, cells are endowed with an alternative mechanism for degrading proteins — autophagy. In

this process, organelles can be degraded regardless of their size or the folding state of their constituent proteins. Many of the components that mediate autophagy have recently been identified as UPR target genes and are important for cells to survive severe ER stress^{60,68}; therefore, as the cell produces more ER to handle an increased protein-folding load, it concomitantly prepares to degrade the organelle and the damaged proteins within it.

Interestingly, during UPR-induced autophagy, ER membranes are selectively sequestered and tightly packaged into autophagosomes. For this reason, the process has been named ER-phagy ('ER eating'), which represents a novel, organelle-selective branch of autophagy⁶⁰. In contrast to other types of autophagy, such as starvation-induced autophagy, the autophagosomes that accumulate in UPR-induced yeast cells do not readily fuse with the vacuole until the stress is abolished, indicating that the sequestration of damaged ER is more important than its ultimate degradation. The mechanisms described above aim to reduce the level of ER stress. However, when the stress is overwhelming, cell-death pathways are activated.

Survival and death of ER-stressed cells

Given the toxic potential of unfolded and misfolded proteins that accumulate in ER-stressed cells, it comes as no surprise that a perturbed and overloaded ER-folding environment is associated with enhanced cell death. However, the molecular mechanisms that are involved in the death of ER-stressed cells remain poorly understood. Calcium from the ER might be involved in the activation of cytoplasmic proteases that contribute to cell death⁶⁹, but it is unknown if and how ER stress promotes such a calcium leak. Similarly, ER stress has been implicated in the activation of various death effectors such as BAK and BAX, which signal through the mitochondria⁷⁰, or caspase-12, which is activated in the cytoplasm^{23,71}. However, the link between perturbation in protein folding in the ER and the activation of these death pathways remains poorly understood (FIG. 5). Furthermore, the available evidence suggests that the same pathways are activated by lethal ER stress and by surmountable ER stress (which does not lead to death). It has been suggested that survival in the face of lower levels of ER stress is facilitated by the intrinsic instability of UPR-induced cell-death mediators such as **CHOP** (C/EBP-homologous protein) and GADD34 (see below). According to this model, the level of these proteins exceeds the death threshold only after prolonged and severe ER stress⁷².

The complexity of the relationship between the UPR and cell survival and cell death is illustrated by considering the role of eIF2 α phosphorylation. Complete loss of PERK-mediated eIF2 α phosphorylation markedly sensitizes cells to death from ER stress^{33,34,73}. However, not all of the effectors of this arm of the UPR contribute to protection; a notable exception is the transcription factor CHOP, which is itself transcriptionally induced by eIF2 α phosphorylation⁵². Deregulated *CHOP* expression promotes cell death⁷⁴, whereas *CHOP* deletion protects against

ER-associated protein degradation

The process whereby misfolded ER proteins are delivered to the cytoplasm for proteasomal degradation.

Autophagy

A collection of pathways by which sections of the cytoplasm, including the organelles suspended in it, are sequestered into membrane-bounded compartments that then fuse with lysosomes, where their content is degraded by acid hydrolases.

Box 2 | Cancer and the unfolded protein response

Malignant transformation and the tumour environment promote endoplasmic reticulum (ER) stress. Tumour ischaemia is one proposed mechanism because protein folding in the ER requires ATP and is sensitive to reduced intracellular glucose⁸⁷. The high mutation load in cancer might also contribute to ER stress by affecting protein folding, and tumour survival is predicted to depend on signalling pathways that maintain chaperone levels to buffer these genetic defects⁸⁸.

The unfolded protein response (UPR) is active in various human tumours⁸⁷. Hypoxia, a common occurrence in solid tumours, is a potent activator of protein kinase RNA (PKR)-like ER kinase (PERK)^{89,90} and its downstream target, activating transcription factor-4 (ATF4)⁹¹. PERK activation was found in transplantable tumours in nude mice⁹² and inositol-requiring protein-1 (IRE1) activity was documented in a human sarcoma tumour xenograft that had been engineered to express an X-box binding protein-1 (XBP1) splicing reporter⁹³.

Several experiments document the contribution of the UPR to tumour survival. Human fibrosarcoma cells with partial knockdown of the ER chaperone immunoglobulin-binding protein (BiP) are impaired in their ability to grow as tumours in nude mice⁹⁴, whereas PERK knockout compromises the ability of Ras-transformed mouse fibroblasts to grow as tumours in nude mice^{92,95}. Similarly, knockdown of XBP1 compromises the growth of transplantable tumours in nude mice⁹⁶. Together, these observations suggest the potential utility of interfering with the UPR as a means of treating cancer.

the death of ER-stressed cells^{75,76}. These observations indicate that *CHOP* evolved to link insurmountable levels of ER stress to the cell-death machinery, and suggest that the fitness of complex metazoans (in which *CHOP* first evolved) is improved by the death of cells that are damaged by ER stress because the dead cells are replaced through regenerative programmes that are developed in long-lived higher eukaryotes⁷⁵. This explanation is favoured by evidence that links *CHOP* to direct effectors of cell death; for example, through repression of *BCL-2* (REF. 74).

However, alternative explanations for increased survival of ER-stressed *CHOP*^{-/-} cells have recently emerged. For example, *CHOP* activates *GADD34*, and reduced levels of *GADD34* protein in *CHOP*^{-/-} cells correlate with sustained elevation of phosphorylated eIF2 α , sustained repression of translation, lower levels of unfolded ER proteins and, consequently, lower levels of ER stress. In keeping with this protective mechanism, *GADD34* deletion also protects cells against death caused by pharmacological agents that perturb protein folding in the ER⁷⁷. Therefore, the *CHOP*-dependent *GADD34*-mediated negative feedback on levels of phosphorylated eIF2 α can, in some circumstances, be maladaptive because it encourages excessive recovery of the ER load in stressed cells.

Further evidence that this example of failed homeostasis arises from inappropriately low levels of phosphorylated eIF2 α is provided by an experiment in chemical genetics. A search for compounds that protect cells against death by exposure to tunicamycin led to salubrinal, a compound that was found to attenuate eIF2 α dephosphorylation and promote higher steady-state levels of phosphorylated eIF2 α (REF. 78). Furthermore, preemptive phosphorylation of eIF2 α (REF. 35) and genetic manipulation to reduce expression of eIF2 (therefore mimicking the effects of its phosphorylation) protected cells against subsequent exposure to ER stress⁷⁹.

These observations are based on experiments using pharmacological agents that severely perturb the folding environment in the ER, but the threshold for activation of *CHOP* (and *GADD34*) might also be set to be inappropriately low in other, more relevant circumstances. For example, *CHOP* deletion protects dopaminergic neurons from the effects of toxins in a model of **Parkinson's disease** that is associated with ER stress⁸⁰, and it also protects insulin-producing β -cells from misfolded insulin in a model of diabetes mellitus⁷⁶. These examples probably report only one side of a delicate balance; in other circumstances, the *GADD34* feedback loop of eIF2 α dephosphorylation and recovery of protein synthesis contributes to the survival of cells that are exposed to agents that induce high levels of PERK activation⁴⁶. Also, in viral infection, elevated eIF2 α phosphorylation probably contributes to organismal survival by promoting apoptosis⁸¹.

The rare human disease of **childhood ataxia with cerebral hypomyelination** presents a particularly interesting example of the potential adverse effects of excessive eIF2 α phosphorylation. The known disease-associated mutations mimic the effects of elevated levels of phosphorylated eIF2 α by reducing the activity of eIF2B, the guanine nucleotide exchange factor that activates the eIF2 complex⁸². It is tempting to speculate that the characteristic episodes of brain damage that are observed in this disease reflect the consequences of minor perturbations that promote ER stress and eIF2 α phosphorylation to levels that are easily tolerated by normal individuals but not by patients with the disease. The need to regulate levels of phosphorylated eIF2 α within narrow bounds has important implications for the possibility of targeting the translational arm of the UPR for therapeutic benefit (BOX 2).

Conclusions and future directions

Despite the advances made in understanding the UPR in recent years, important unanswered questions remain. The structural basis for recognition of the upstream stress signal by the ER stress transducers has only begun to be unravelled and we have only a limited understanding of the steps that are involved in activation of IRE1, PERK and ATF6 effector functions. Our understanding of the quantitative aspects of gene activation in the UPR is similarly rudimentary and we have only vague ideas of how the UPR interfaces with death and differentiation decisions or how it connects to parallel stress pathways such as autophagy. Despite these limitations, we can begin to consider the implications of manipulating signalling in the UPR. This is a worthwhile endeavour because the upstream signalling components of both the IRE1 and PERK arms of the pathway are protein kinases; therefore, drugs might be found that can target them selectively.

The UPR protects cells against normal and unusual levels of ER stress by enhancing the capacity of the secretory apparatus and by reducing ER load. Different cells are predicted to have different levels of sensitivity to ER stress and, thus, will exhibit different levels of tolerance to inhibition of PERK or IRE1. Cancer cells might be

Ischaemia

The metabolic consequences of inadequate blood supply. It is a common occurrence in large tumours and is associated with a poor response to treatment.

Nude mouse

An immune-compromised mouse that tolerates tumour xenografts and is used to model tumour growth in a living animal.

especially sensitive to such manipulation (BOX 2), but the width of the therapeutic window between hypersensitive cancer cells and the susceptible, relevant normal tissues remains unknown.

Various human illnesses are caused by mutations that reduce the expression of essential membrane or secreted proteins below a certain disease threshold. Despite the fact that many such mutations have only subtle effects on folding and can be tolerated in functional proteins, the mutations are nonetheless sufficiently severe to cause retention and degradation of the mutant protein in the ER. The UPR directly regulates the expression of the chaperones and degradation machinery that constitute the ER quality control system that retains attempts to fold but, ultimately, destroys most such mutant proteins⁸³. The health of patients with such diseases would be served by loosening the stringency of ER quality control, a goal

that might be attained by inhibiting portions of the UPR. In other circumstances, a mutation might cause a protein to misfold into a dangerous conformation and patient health would then be served by enhancing retention in the ER and degradation of the mutated protein⁸⁴. Better tools are needed to predict how tweaking the UPR might affect the handling of mutant proteins in a variety of circumstances.

Beyond these relatively well-defined goals, we also need to assess critically the role of ER stress in myriad other pathophysiological conditions that are not associated with the expression of any known specific mutant protein. In that context, special attention needs to be paid to the intersection between the UPR and other signalling pathways, even if we still lack an intuitive understanding of how these pathways are linked to cellular adaptation to the unfolded protein load in the ER.

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Acknowledgments

We are indebted to our laboratory members and trainees who contributed ideas and experiments to many of the studies described here, and we apologize to those colleagues whose publications could not be cited owing to space limitations. Work in our laboratories is supported by the National Institutes of Health (D.R. and P.W.) and the Howard Hughes Medical Institute (P.W.).

Competing interests statement

The authors declare no competing financial interests.

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