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Endoplasmic reticulum stress response in yeast and humans

Haoxi WU*^{†1}, Benjamin S. H. NG*¹ and Guillaume THIBAUT*^{‡2}

*School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore

†College of Life Sciences, Sichuan University, Chengdu 610064, China

‡Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 308232, Singapore

Synopsis

Stress pathways monitor intracellular systems and deploy a range of regulatory mechanisms in response to stress. One of the best-characterized pathways, the UPR (unfolded protein response), is an intracellular signal transduction pathway that monitors ER (endoplasmic reticulum) homeostasis. Its activation is required to alleviate the effects of ER stress and is highly conserved from yeast to human. Although metazoans have three UPR outputs, yeast cells rely exclusively on the Ire1 (inositol-requiring enzyme-1) pathway, which is conserved in all Eukaryotes. In general, the UPR program activates hundreds of genes to alleviate ER stress but it can lead to apoptosis if the system fails to restore homeostasis. In this review, we summarize the major advances in understanding the response to ER stress in *Sc* (*Saccharomyces cerevisiae*), *Sp* (*Schizosaccharomyces pombe*) and humans. The contribution of solved protein structures to a better understanding of the UPR pathway is discussed. Finally, we cover the interplay of ER stress in the development of diseases.

Key words: ER stress, IRE1, lipid disequilibrium, protein homeostasis, unfolded protein response, UPR-related diseases. The UPR (unfolded protein response) is activated to alleviate the effects of ER (endoplasmic reticulum) stress and is highly conserved from yeast to human. In this review, we summarize the major advances in yeast and humans.

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INTRODUCTION

Stress pathways respond to systemic perturbations by regulating diverse functions. They are specialized mechanisms designed to monitor and maintain intracellular homeostasis. A response targeted to restore homeostasis in the ER (endoplasmic reticulum), the UPR (unfolded protein response), is one of the best-studied cellular stress responses. Upon ER stress, three independent branches sense stress, with the Ire1 (inositol-requiring enzyme-1) branch being the most highly conserved among eukaryotes. In yeast, only the Ire1 pathway is found while metazoans utilize two additional pathways, double-stranded RNA-activated PERK (protein kinase-like ER kinase) and ATF6 (activating tran-

scription factor 6) (Figure 1). In general, these ER-localized transmembrane proteins sense ER stress resulting in the activation of their respective pathways. Translational attenuation and activation of UPR target genes are the most common UPR outputs found in eukaryotes. However, the UPR programme can lead to apoptosis if cells fail to reach homeostasis and undergo prolonged stress.

Early work on the UPR were done in animal cells, where the expression of ER-resident molecular chaperones [BiP (immunoglobulin heavy-chain-binding protein)/GRP78 (glucose-regulated protein 78), GRP94, PDI (protein disulphide isomerase)/ERp59 and ERp72] were shown to be induced by different treatments causing the accumulation of unfolded proteins in the ER [1,2]. The major breakthrough, reporting the

Abbreviations: AD, Alzheimer's disease; ATF6, activating transcription factor 6; BiP, immunoglobulin heavy-chain-binding protein; bZIP, basic leucine zipper; CHOP, C/EBP homologous protein; cIAPs, cellular inhibitors of apoptosis proteins; cLD, core luminal domain; eIF2 α , eukaryotic initiation factor 2 α ; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GADD34, growth-arrest and DNA-damage-inducible 34; GRP78, glucose-regulated protein 78; HD, Huntington's disease; HSP70, heat-shock protein 70; Ire1, inositol-requiring enzyme-1; KEN, kinase-extension nuclease; MHC, major histocompatibility complex; NAFLD, non-alcoholic fatty liver disease; PD, Parkinson's disease; PDI, protein disulphide isomerase; PERK, protein kinase-like ER kinase; PKR, protein kinase R; PS, presenilin; RIDD, regulated Ire1-dependent decay; *Sc*, *Saccharomyces cerevisiae*; *Sp*, *Schizosaccharomyces pombe*; UGT, UDP-glucose-glycoprotein glucosyltransferase; UPR, unfolded protein response.

¹ Both the authors contributed equally to this work.

² To whom correspondence should be addressed (email thibault@ntu.edu.sg).

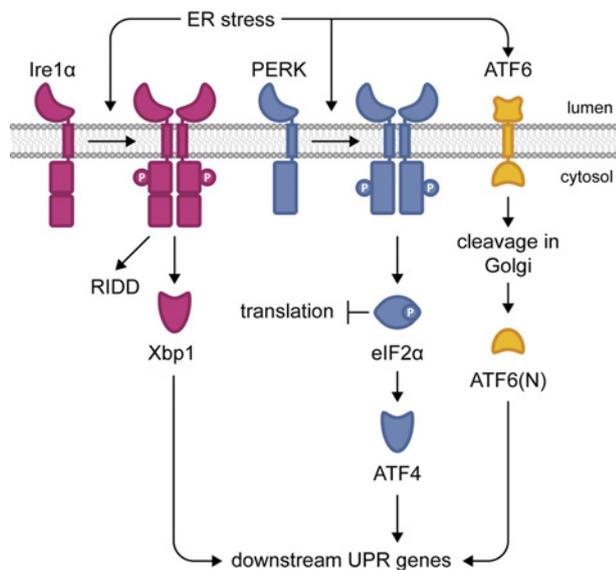


Figure 1 Activation of the UPR

ER stress transducer, Ire1 α , PERK and ATF6 form the three branches of the UPR pathways in mammals. In response to ER stress, the release of the molecular chaperone BiP from the luminal domain of Ire1 α , promotes binding of misfolded proteins. Subsequently, Ire1 α oligomerizes and phosphorylates itself, splices *XBP1* mRNA and this results in the translation of the transcription factor XBP1 regulating downstream signalling cascade. Upon prolonged ER stress, Ire1 α cleaves mRNAs to relieve protein load through its RIDD activity. PERK oligomerizes and phosphorylates itself together with eIF2 α , where it attenuates protein translation. It further activates the transcription factor ATF4, which carries out downstream activation of UPR genes. Under ER stress, ATF6 is packaged into vesicles and transported to the Golgi apparatus. Cleavage of ATF6 luminal and transmembrane domain occur, where the N-terminal cytosolic fragment, ATF6(N) localize into the nucleus to activate UPR target genes.

missing elements in the UPR pathway from unfolded proteins to the activation of UPR-specific genes, was done in *Sc* (*Saccharomyces cerevisiae*). Upon accumulation of unfolded proteins, *HAC1* mRNA was found to be spliced by Ire1 protein, and only the spliced form results in translation of stable transcription factor Hac1 protein [3–5]. These findings opened the door to many new discoveries in understanding the UPR come from all model organisms, this review will focus on the discoveries culminating from budding yeast, fission yeast and mammals, as well as diseases related to the UPR.

ACTIVATION OF THE UPR IN YEAST

In *Sc*, ER stress is monitored by the transmembrane sensor protein Ire1 (Figure 2A). Ire1 is activated by either direct binding of unfolded proteins, or from the release of the molecular chaperone BiP from the luminal domain of Ire1. Both mechanisms of activation have been proposed by different groups and will be dis-

cussed in detail in the Ire1 structure section. Upon activation, Ire1 oligomerizes followed by *trans*-autophosphorylation through its cytosolic kinase domain [6]. When activated, the cytosolic ribonuclease domain of Ire1 cleaves the intron of pre-messenger RNA *HAC1* to initiate synthesis of Hac1 transcription factor [3–5]. Hac1 then translocates into the nucleus to regulate the expression of UPR target genes. The UPR can alleviate stress by reversing severe dysfunctions through the up-regulation of nearly 400 target genes [7]. These target genes include ER chaperones, lipid biosynthesis enzymes and ERAD (ER-associated degradation) machinery. The UPR program appears to be adaptable and might be remodelled differently according to the needs of the cells. This differential regulation of the UPR, from different stressors, suggests the involvement of additional unidentified regulatory factors [7].

The UPR-related protein Ire1 is conserved in *Sp* (*Schizosaccharomyces pombe*) [8] (Figure 2B). Despite having no orthologues of *HAC1* or *XBP1* mRNA being identified, Ire1 still plays an important role to alleviate ER stress [9,10]. Unlike *Sc* Ire1, activated *Sp* Ire1 degrades ER-localized mRNAs to relieve protein load in a pathway called RIDD (regulated Ire1-dependent decay). This pathway was first identified in metazoan where Ire1 degrades mRNAs in addition to *XBP1* splicing [11,12]. Surprisingly, certain mRNAs cleaved by *Sp* Ire1 are stabilized instead of being degraded [10]. For example, *BIP1* mRNA, which encodes an HSP70 (heat-shock protein 70) family protein, is recognized and cleaved by Ire1, but it remains stable and its translation is increased. Notably, it was reported that a *BIP1* mRNA mutant, which is no longer cleaved by Ire1 in *Sp*, exhibits lower viability during ER stress. Other players are likely to work in synergy with Ire1 to regulate the UPR pathway. The UGT (UDP-glucose-glycoprotein glucosyltransferase) (*gpt1*) and a calnexin orthologues (*cnx1*) have been identified similarly with Ire1, from a genetic screen, to alleviate ER stress [9]. UGT was first identified from rat liver extract to recognize only misfolded proteins and to be essential under extreme ER stress in *Sp* [13,14]. The other identified protein from the screen, Cnx1, promotes folding of glycosylated proteins in the ER and may cooperate with BiP1 [15]. The first 160 residues of Cnx1 in *Sp* was found to be sufficient for cell survival under normal conditions [16,17]. Reinforcing its link to ER stress, *Sp* Cnx1 promotes apoptosis which is mediated by Ire1 [18]. Thus, the recent development in our understanding of ER stress response in *Sp* seems unique and it might help in elucidating the similar pattern in higher organisms.

THE UPR ACTIVATION IN MAMMALS

In mammals, the presence of three different ER stress transducers facilitates the activation of the UPR [19] (Figure 1). Two forms of IRE1 are found in mammals where IRE1 α is expressed ubiquitously while IRE1 β solely in the intestinal and lung epithelium [20,21]. In human, IRE1 α and IRE1 β proteins are encoded

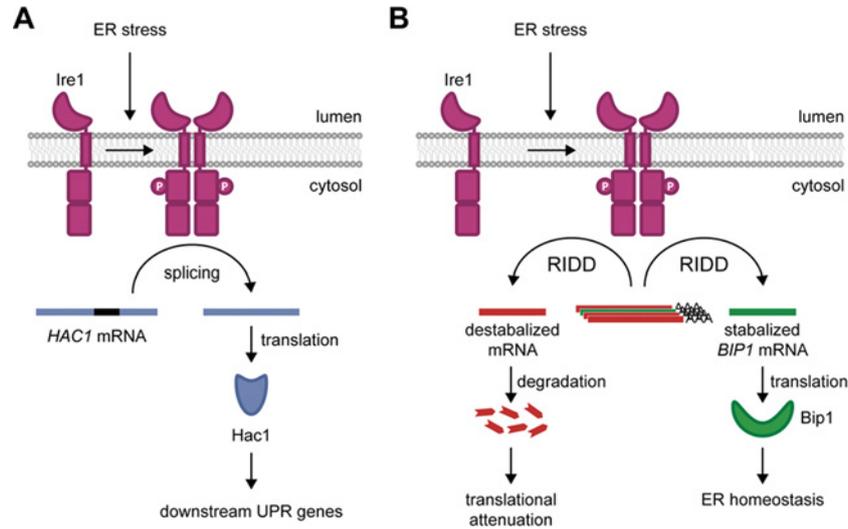


Figure 2 UPR in yeast

(A) *S. cerevisiae* Ire1 is activated by ER stress. Upon activation, Ire1 undergoes *trans*-autophosphorylation and oligomerization. *HAC1* mRNA is spliced by activated Ire1 through its RNase domain. Upon translation, transcription factor Hac1 up-regulates UPR target genes to restore homeostasis. (B) In *S. pombe* upon ER stress, Ire1 triggers downstream RIDD; the protein BiP1's poly-A tail is recognized and cleaved by RIDD, but BiP1 protein is stabilized despite the cleavage of its mRNA. BiP1 protein is important for cell survival during stress condition; there are possible unknown candidates also involved in stress response, either in ER lumen or on ER membrane.

by *ERN1* and *ERN2* genes, respectively. Ire1 α branch of the UPR has been well studied because of its ubiquitous-expressing nature. The transcription factor downstream of Ire1 α , XBP1, exhibits variance in its primary amino acid sequence with Hac1 but shares the common Ire1-mediated unconventional splicing activation of its mRNA and the bZIP (basic leucine zipper) motif. XBP1 activates similar downstream target genes as Hac1 in *Sc*, with the induction of genes involved in protein folding as well as in the secretory pathway [22]. With considerable redundancies existing between the three UPR pathways, IRE1–XBP1 pathway is dispensable to the activation of major chaperones such as BiP and GRP94 [23], but still plays crucial roles in ER homeostasis and metabolic pathways [24].

PERK, which is an ER transmembrane kinase, mediates transcriptional and translational control of the UPR program [25]. Upon ER stress, PERK oligomerizes and phosphorylates itself together with eIF2 α (eukaryotic initiation factor 2 α) (Figure 1). eIF2 α phosphorylation results in temporary attenuation of the overall protein translation and up-regulation of the transcription factor ATF4. This translation inhibition resultantly decreases the influx of proteins entering the ER, reducing ER protein folding load and alleviating ER stress. The absence of the PERK signalling cascade in *Sc* disallows the regulation of easing ER stress when protein integrity is compromised, where continued protein synthesis occurs even under ER stress. Paradoxically, certain mRNA are preferentially translated when eIF2 α is limiting, one of which is transcription factor ATF4. Subsequently, ATF4 up-regulates CHOP (C/EBP homologous protein) and GADD34 (growth-arrest and DNA-damage-inducible 34). CHOP promotes ER stress-induced apoptosis [26] while GADD34 is involved in

a negative feedback loop to counteract PERK by dephosphorylation of eIF2 α , which resumes protein synthesis and sensitizes cells to apoptosis [27]. Interestingly, PERK also inhibits ER stress-induced apoptosis via induction of cIAPs (cellular inhibitors of apoptosis proteins) [28], and is a critical crosstalk regulator to influence the entire UPR in determining cell fate under ER stress [29]. In addition, another study has shown that activation of PERK could lead to down-regulation of anti-apoptosis protein XIAP, which could lead to increase in apoptosis [30]. These reports suggest that even when facing similar stress, cells could respond to it differently, and other factors might be involved to regulate how cells respond to various stresses, which could ultimately determine cell fate.

Upon detection of unfolded protein accumulation, ATF6 is packaged into vesicles and transported to the Golgi apparatus [31]. Cleavage of ATF6 luminal and transmembrane domain occurs subsequently by S1P and S2P proteases, liberating the N-terminal cytosolic fragment, ATF6(N), for localization into the nucleus to activate UPR target genes [32]. A vast array of genes are activated downstream of ATF6(N), most noticeably BiP, PDI, and GRP94. Additional studies have shown that ATF6(N) is a major inducer for downstream response of ER chaperones and ERAD components [33,34].

Recent efforts to understand the involvement of factors participating in the UPR have shown little progress. Being intimately intertwined together, it would be hard to investigate the effect of individual factors, where compensation by other UPR branches could set in. The UPR has also been shown to be tightly linked to ERAD as well as lipid regulation [35], hence there is difficulty in isolating and understanding the UPR in cell physiology.

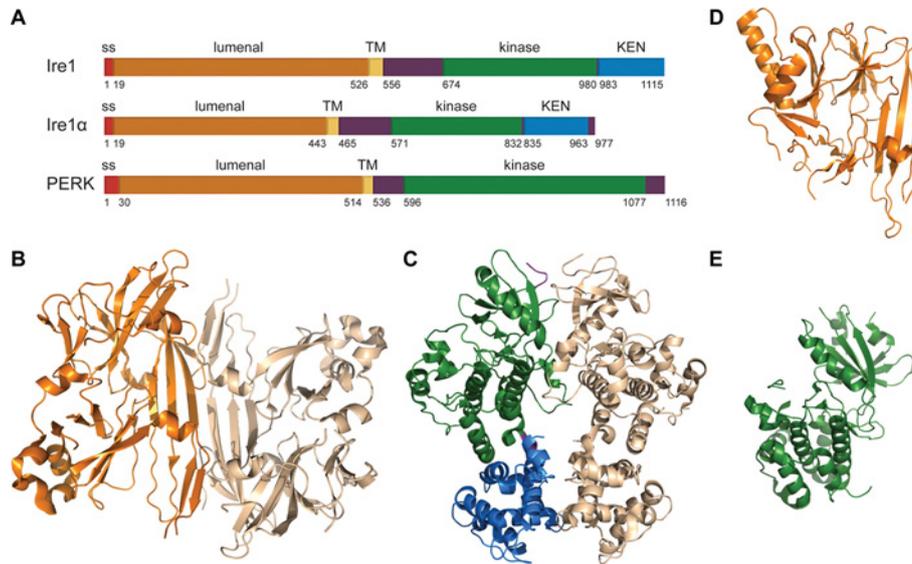


Figure 3 Solved protein structures of the UPR activation pathway

(A) Schematic representation of yeast Ire1, human Ire1 α and mouse PERK proteins. Ss (Signal sequence), TM (transmembrane domain), KEN domain. (B) Ribbons representation of dimerized cLD (amino acids 111–449) of *Sc* (*S. cerevisiae*) Ire1 [PDB (Protein Data Bank) code: 2BE1]. (C) Ribbons representation of the dimerized cytosolic domain of *Sc* Ire1 (PDB code: 3FBV). (D) Ribbons representation of human Ire1 α luminal domain. The dimer adapts a back to back orientation (PDB code: 2HZ6). (E) Ribbons representation of PERK protein kinase domain from mouse (PDB code: 3QD2). All structures were drawn using PyMOL (www.pymol.org).

UPR ACTIVATED FROM LIPIDS

Many lipid synthesis genes are up-regulated from the UPR activation programme [36], indicating interconnection between lipid composition and ER stress. Inositol, which is an important regulator of lipid metabolism in yeast, is regulated by the UPR [37,38]. Moreover, deletion of fatty acid and sphingolipid biosynthesis genes such as *SUR4* and phospholipid synthesis genes such as *OPI3* or *INO1* activate the UPR [39,40]. The UPR can control lipid synthesis genes in order to balance membrane lipid composition through the IRE1–RIDD pathway [41]. Changes in membrane lipid composition may lead to the activation of the UPR. Previously, we demonstrated that the UPR remodels protein homeostasis network, in yeast cells, instead of restoring lipid composition under global lipid disequilibrium [40]. Lipid imbalance also contributes to the disruption of calcium homeostasis in mammals [42]. Other than causing accumulation of misfolded proteins, calcium metabolic imbalance results in the accumulation of free fatty acids causing the activation of the UPR [43]. Alternately, membrane lipid composition imbalance can be directly sensed by Ire1 protein resulting in UPR activation. An *Sc* Ire1 mutant failing to bind unfolded proteins, bZIP Ire1, was observed to activate the UPR normally during lipid imbalance [44]. This study suggested, for the first time, that Ire1 can sense stress from its transmembrane or cytosolic domains. More recently, a novel mechanism of UPR activation, during lipid perturbation, was proposed in mammalian cells [45]. Ire1 α and PERK proteins lacking their luminal domain sense saturated lipids and they are sufficient

to activate the UPR in mammalian cells (Figure 3A). Both activators undergo phosphorylation, and activate their downstream pathways similarly to their respective full-length protein. In addition, PERK was shown to directly sense exogenous saturated fatty acid by *in vitro* liposome reconstitution [45]. Thus, lipid and protein metabolisms are tightly regulated and share common regulatory pathways.

IRE1 STRUCTURE AND ACTIVATION

To better understand the pathways leading to UPR activation, substantial efforts have been made to solve protein structures, in particular the ER stress transducers. However, due to the variable hydrophobicity within membrane proteins and the difficulty to solubilize them, it has been challenging to obtain structures at high resolution [46]. The first reported structure of UPR transactivator was the cLD (core luminal domain, amino acids 111–449) of Ire1, from *Sc* at 2.98 Å resolution [47] (Figure 3B). cLD dimer exhibits a groove similar to MHC (major histocompatibility complex). From this observation, it was proposed that the direct binding of unfolded proteins to Ire1 luminal domain is sufficient to activate the UPR in yeast.

Before acquisition of the Ire1 luminal domain structure in *Sc*, it was accepted that Ire1 is activated by the release of Hsp70 molecular chaperone BiP from its luminal domain. The importance of ER-resident Hsp70 family proteins, during stress conditions,

was reported by several groups where they are up-regulated under ER stress [1,48,49]. Moreover, overexpression of BiP is sufficient to attenuate the UPR to stress [50]. In *Sc*, BiP was reported to serve as a negative regulator of the UPR [51]. BiP keeps Ire1 inactive by directly binding its luminal domain but during ER stress, it dissociates to bind unfolded proteins resulting in Ire1 activation.

However, several other studies contradict this model and support the direct binding model. An *Sc* Ire1 luminal domain deletion mutant was generated to disrupt BiP binding [52]. In this mutant, the UPR activation level appeared normal during ER stress. It indicates that BiP Ire1 interaction is not required to activate Ire1. Thus, the dissociation of BiP and self-association of Ire1 is not sufficient to activate the UPR suggesting that more steps are involved in the activation pathway [53]. Interestingly, several studies reported that Ire1 directly binds unfolded proteins and prevent protein aggregate formation [44,54–56]. In addition, Ire1 preferentially binds to peptides enriched in basic and hydrophobic residues reinforcing its affinity for misfolded proteins (Gardner and Walter[54], #23). The binding pattern was proposed to be like ligand–receptor interaction in which BiP serves by preventing Ire1 oligomerization [49].

The cytosolic domain of Ire1 has two functional domains with kinase and RNase enzymatic activities [57] (Figure 3A). Structural studies of Ire1 cytosolic domain helped in dissecting the cascade of events of the UPR activation. The first crystal structure of Ire1 cytosolic domain was at 2.4 Å resolution [58] (Figure 3C). Based on its motif folding, RNase domain was redefined as the KEN (kinase-extension nuclease) domain. KEN domain possesses ribonuclease activity after *trans*-autophosphorylation of its adjacent kinase domain. Interestingly, there is no known protein other than Ire1 with both kinase and RNase activities. Ire1 KEN domain is similar to mammalian RNase L, which cleaves endogenous and viral RNA within the cell upon activation from interferon [59,60]. Four key residues (Y1043, R1056, N1057 and H1061) of Ire1 form the RNase active site and they are conserved in RNase L [58]. The oligomerization of Ire1 cytosolic domain is essential to its enzymatic activity, which is driven by the oligomerization of Ire1 luminal domain [61]. Together with the structural information of Ire1's RNase inactive mutant [62], it was revealed that the kinase activity of Ire1 is not required during the UPR activation cascade but essential in Ire1 deactivation [63]. On the contrary, RNase activity is essential for the same process.

In both yeast and human, dimerized Ire1 form MHC-like grooves [64]. However, the Ire1 α groove is thought to be too small to accommodate unfolded proteins (Figure 3D). Gln105 of Ire1 α , which forms hydrogen bond to adjacent residues, prevents direct binding of unfolded proteins. Thus, it supports the conventional indirect UPR activation model. In this model, BiP dissociates from Ire1 α and triggers its oligomerization and phosphorylation. After activation, Ire1 α adopts a face-to-face orientation when its phosphorylation is prevented with ATP competitor sunitinib [65]. In contrast, dimerized *Sc* Ire1 RNase domain holds a back-to-back orientation when activated. As a result, face-to-face orientation of Ire1 α would compromise the activation of the UPR by failing to splice *XBPI*. The different activation mech-

anism of Ire1 α luminal domain, compared with *Sc* Ire1, might confer greater specificity in responding to ER stress and thus complementing the other two branches of the UPR. It is worth mentioning that there is still no full-length structure of Ire1 reported yet. Structural information of Ire1 transmembrane domain would be valuable to give us a better understanding of its role in sensing lipid perturbation [44,45].

PERK AND ATF6 STRUCTURES

Recently, the structure of PERK's kinase domain was solved at 2.8 Å resolution [66] (Figure 3E). Upon activation of PERK, its cytosolic kinase domain adapts a back-to-back dimer structure, and its dimerization is driven by PERK ER lumen domain dimerization. This further activates PERK's kinase domain resulting in *trans*-autophosphorylation [57]. *Trans*-autophosphorylation of PERK activation loop (residues 953–990) is indispensable for its activity [66]. PERK kinase domain shares sequence similarity with PKR (protein kinase R). It was reported before that PKR phosphorylates eIF2 α at Ser⁵¹, leading to inhibition of translation [67]. Thus, it is not surprising that activated PERK also phosphorylates eIF2 α to alleviate ER stress [66].

The 3D structure of ATF6 remains to be elucidated. ATF6 is synthesized as a precursor protein (p90ATF6), which is localized at the ER in unstressed cells. In response to ER stress, ATF6 is packaged into vesicles and transported to the Golgi apparatus and the N-terminal fragment is cleaved off (p50ATF6) [31,32].

UPR AND DISEASES

Proteins are the key regulators of cellular diversity in function, performing numerous roles such as enzymatic reactions, mechanical support, transport of substrates and signal transduction. The UPR maintains the integrity of protein synthesis and alleviates ER stress through the regulation of various proteins. Hence, UPR signalling is involved deeply in numerous physiological processes besides protein quality control [68]. Several diseases such as diabetes, NAFLD (non-alcoholic fatty liver disease), cystic fibrosis, PD (Parkinson's disease), HD (Huntington's disease), AD (Alzheimer's disease), inflammation, cancer and liver failure are associated with the UPR pathways [69] (Figure 4). Implications of the UPR in metabolic diseases, cancer and neurodegenerative diseases will be reviewed in this section.

NAFLD is an emerging and now the most common cause of chronic liver enzyme elevations and cryptogenic cirrhosis because of the prevalence of obesity [70]. The failure of the UPR to rapidly re-establish ER homeostasis via genetic ablation of *eIF2 α* , *IRE1 α* or *ATF6 α* results in hepatic steatosis, where the capacity to oxidize fatty acids is impaired [71]. This is further exacerbated by dysfunction in the lipoprotein secretion pathway.

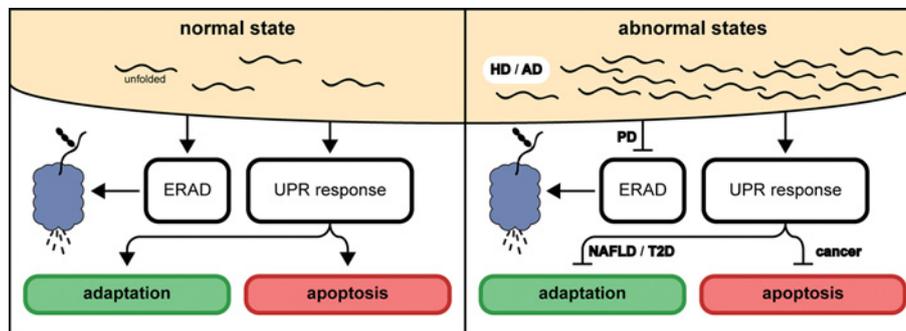


Figure 4 Diseases linked to ER stress

During ER stress, misfolded proteins arising in the ER is assisted with chaperones for refolding, and failure to be refolded to their native state would result in their degradation via the ERAD pathway. The UPR is activated with the accumulation of unfolded or misfolded proteins, which would then halt protein translation and induce stress-response genes. Under prolonged ER stress, apoptosis is initiated by the UPR. Diseased states often arise from the failure of the UPR to respond well under ER stress, or from an accumulation of unfolded proteins. Inadequate response of the UPR could result when elements in the UPR signalling cascade is down-regulated and hence, a sufficient response could not be mounted to alleviate ER stress. Diseases such as NAFLD, T2D (type II diabetes) and cancer are implicated in this model. Mutations in protein coding genes could cause proteins synthesized to be misfolded and form aggregates rapidly. This could be severe that the ERAD fails to degrade the proteins adequately and the UPR is unable to compensate for the ER stress. This model often includes degenerative diseases such as PD, HD and AD.

It was reported in another study that heterozygous ATF4 mice benefitted in protection from diet-induced obesity and diet-induced hepatic steatosis [72]. Overexpression of mediators involved in UPR such as *GRP78* improves insulin action and hepatic steatosis [73]. Other metabolic diseases such as type 2 diabetes could also be the consequence of ER dysregulation. ER stressor deficiency in XBP1 induces the development of insulin resistance [24], where body cells fail to respond to insulin in uptaking glucose. In other studies to reveal the mechanism of how ER stress develops in obesity, the authors have found that free fatty acids could be the trigger of ER stress via perturbation of ER membrane integrity [74,75]. Increased mTOR (mammalian target of rapamycin) signalling pathway, which regulates cell proliferation and survival, is also found in most obese patients to block insulin signalling pathways [76]. Additionally, disruption of signalling between p85s and XBP1 occurs in obesity, where p85 serves to activate XBP1 after insulin stimulation, resulting in decreased localization of XBP1 to the nucleus for the activation of the UPR [77]. Thus, the UPR appears deeply intertwined with lipid homeostasis, where dysregulation in the UPR is often manifested in various metabolic diseases.

Neurodegenerative diseases frequently arose from the accumulation of misfolded proteins, leading to the death and loss of neurons necessary for physiological functions [78,79]. In PD, the *Parkin* gene which encodes an E3 ubiquitin ligase is mutated and results in the failure of substrate degradation. This subsequently leads to unfolded protein accumulation, causing UPR activation and eventually apoptosis of neurons through the action of CHOP [80,81]. The accumulation of abnormally long huntingtin protein from the increase in CAG nucleotide repeats in the *huntingtin* gene results in HD [82]. Accumulation of aggregates impairs the proteasome degradation system, contributing to the accumulation of other misfolded proteins and subsequently triggering ER stress [83]. Other evidence such as elevated expression of UPR target

genes; *CHOP*, *GRP78* and *Herp* are found in patients with HD [84], and perturbation of ER calcium homeostasis is linked to a mutant *huntingtin* gene [85]. This suggests that the mutant *huntingtin* continuously activates UPR target genes and have wide spread consequences on other facets of ER homeostasis. AD is characterized by aggregation of fibrous insoluble proteins, commonly the amyloid- β peptide [86]. Accumulation of unfolded proteins, elevated ER stress, and activation of the UPR are commonly found in many cases of patients with AD [87,88], compelling a model where there is accumulation of misfolded proteins instead of dysregulation of the UPR. PS (presenilin) are highly conserved transmembrane proteins which regulates the cleavage of other proteins at their transmembrane domain, and is involved in ER calcium trafficking and homeostasis [89]. They are revealed to have as many as 100 mutation variations in familial AD, signifying the importance of PS in its contribution to AD. Neurons are particularly sensitive to toxic aggregate-prone proteins, and hence neurodegenerative disease often arises from the buildup of such protein plaques. The accumulation of aggregates in the cytosol could indicate similar approaches in therapeutic treatment, and further understanding of the involvement of the UPR in the development of the disease could provide clues.

The involvement of the UPR in tumorigenesis is intimate yet complex. Following the onset of malignancy and rapid tumour growth, inadequate vascularization could subsequently result in microenvironmental stress such as hypoxia and depletion of nutrients. Additionally, tumour-intrinsic stress factors such as defects in synthesis of biomolecules arising from mutations in the genes could elevate ER stress further [90,91]. The paradox of the UPR in tumorigenesis begins, where UPR up-regulates protein folding capacity and ensures the continued integrity of protein folding, upholding cell survival [92]. The UPR has been shown to contribute in this fashion to protect tumorigenic cells from undergoing apoptosis under hypoxia conditions [93]. *GRP78* is

found as a critical factor in promoting cancer; increasing proliferation, avoiding apoptosis and the promotion of angiogenesis [94]. However, when chronic ER stress sets in, failure to restore ER homeostasis causes the UPR to activate the apoptotic cascade in tumour cells. CHOP is a key regulator [95] but not the sole factor [26] involved in inducing apoptosis, in which the alteration of several genes by CHOP leads to activation of various apoptotic pathways [92,96].

CONCLUSION AND PERSPECTIVE

The recognition that the UPR is deeply associated with various diseases, especially in metabolic and neurodegenerative diseases, allows us to appreciate the UPR pathways better. However, interactions between the three branches are not well characterized, and more has to be done in order to elucidate a more detailed mechanistic understanding of the UPR elements. This would allow for the effective manipulation of factors involved in the UPR, which could prove extremely valuable in developing therapeutic treatments for such diseases. While there is no universal treatment, it will be useful to draw parallel approaches to treatment when diseases are found to share conspicuous characteristics or causes. Identifying and targeting the UPR elements, which fail to perform before the development of chronic ER stress would be ideal. Induction of such UPR factors naturally or artificially via exogenous means could prevent the progression of UPR-related diseases. In less preferred but still beneficial cases, artificial means such as chemical chaperones [76] could ease mild ER stress or prolong the onset of diseases. Alleviating ER stress via increasing ER-folding capacity could be beneficial for obesity or cystic fibrosis [76] by increasing insulin sensitivity or reducing the secretion of mutated proteins. In other circumstances, targeting the UPR to promote the apoptotic cascade would be ideal for diseases such as in cancer and TSC (tuberous sclerosis complex) [92,97]. Novel methodological approaches in regulating or influencing the UPR would allow greater flexibility and sensitivity to be applied to various diseases implicated by the UPR.

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REFERENCES

- Kozutsumi, Y., Segal, M., Normington, K., Gething, M. J. and Sambrook, J. (1988) The presence of malformed proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature* **332**, 462–464 [CrossRef PubMed](#)
- Dorner, A. J., Wasley, L. C., Raney, P., Haugejorden, S., Green, M. and Kaufman, R. J. (1990) The stress response in Chinese hamster ovary cells. Regulation of ERp72 and protein disulfide isomerase expression and secretion. *J. Biol. Chem.* **265**, 22029–22034 [PubMed](#)
- Cox, J. S., Shamu, C. E. and Walter, P. (1993) Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. *Cell* **73**, 1197–1206 [CrossRef PubMed](#)
- Mori, K., Ma, W., Gething, M. J. and Sambrook, J. (1993) A transmembrane protein with a *cdc2+*/CDC28-related kinase activity is required for signaling from the ER to the nucleus. *Cell* **74**, 743–756 [CrossRef PubMed](#)
- Cox, J. S. and Walter, P. (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* **87**, 391–404 [CrossRef PubMed](#)
- Shamu, C. E. and Walter, P. (1996) Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the endoplasmic reticulum to the nucleus. *EMBO J.* **15**, 3028–3039 [PubMed](#)
- Thibault, G., Ismail, N. and Ng, D. T. (2011) The unfolded protein response supports cellular robustness as a broad-spectrum compensatory pathway. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 20597–20602 [CrossRef PubMed](#)
- Wood, V., Gwilliam, R., Rajandream, M. A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S. et al. (2002) The genome sequence of *Schizosaccharomyces pombe*. *Nature* **415**, 871–880 [CrossRef PubMed](#)
- Frost, A., Elgort, M. G., Brandman, O., Ives, C., Collins, S. R., Miller-Vedam, L., Weibezahn, J., Hein, M. Y., Poser, I., Mann, M. et al. (2012) Functional repurposing revealed by comparing *S. pombe* and *S. cerevisiae* genetic interactions. *Cell* **149**, 1339–1352 [CrossRef PubMed](#)
- Kimmig, P., Diaz, M., Zheng, J., Williams, C. C., Lang, A., Aragon, T., Li, H. and Walter, P. (2012) The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis. *Elife* **1**, e00048 [CrossRef PubMed](#)
- Hollien, J. and Weissman, J. S. (2006) Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* **313**, 104–107 [CrossRef PubMed](#)
- Hollien, J., Lin, J. H., Li, H., Stevens, N., Walter, P. and Weissman, J. S. (2009) Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J. Cell Biol.* **186**, 323–331 [CrossRef PubMed](#)
- Sousa, M. and Parodi, A. J. (1995) The molecular basis for the recognition of misfolded glycoproteins by the UDP-Glc:glycoprotein glucosyltransferase. *EMBO J.* **14**, 4196–4203 [PubMed](#)
- Fanchiotti, S., Fernandez, F., D'Alessio, C. and Parodi, A. J. (1998) The UDP-Glc:Glycoprotein glucosyltransferase is essential for *Schizosaccharomyces pombe* viability under conditions of extreme endoplasmic reticulum stress. *J. Cell Biol.* **143**, 625–635 [CrossRef PubMed](#)
- Jannatipour, M., Callejo, M., Parodi, A. J., Armstrong, J. and Rokeach, L. A. (1998) Calnexin and BiP interact with acid phosphatase independently of glucose trimming and reglucosylation in *Schizosaccharomyces pombe*. *Biochemistry* **37**, 17253–17261 [CrossRef PubMed](#)
- Jannatipour, M. and Rokeach, L. A. (1995) The *Schizosaccharomyces pombe* homologue of the chaperone calnexin is essential for viability. *J. Biol. Chem.* **270**, 4845–4853 [CrossRef PubMed](#)
- Hajjar, F., Beauregard, P. B. and Rokeach, L. A. (2007) The 160 N-terminal residues of calnexin define a novel region supporting viability in *Schizosaccharomyces pombe*. *Yeast* **24**, 89–103 [CrossRef PubMed](#)
- Guerin, R., Arseneault, G., Dumont, S. and Rokeach, L. A. (2008) Calnexin is involved in apoptosis induced by endoplasmic reticulum stress in the fission yeast. *Mol. Biol. Cell* **19**, 4404–4420 [CrossRef PubMed](#)



- 19 Walter, P and Ron, D. (2011) The unfolded protein response: from stress pathway to homeostatic regulation. *Science* **334**, 1081–1076 [CrossRef PubMed](#)
- 20 Bertolotti, A., Wang, X., Novoa, I., Jungreis, R., Schlessinger, K., Cho, J. H., West, A. B. and Ron, D. (2001) Increased sensitivity to dextran sodium sulfate colitis in IRE1beta-deficient mice. *J. Clin. Invest.* **107**, 585–593 [CrossRef PubMed](#)
- 21 Martino, M. B., Jones, L., Brighton, B., Ehre, C., Abdulah, L., Davis, C. W., Ron, D., O'Neal, W. K. and Ribeiro, C. M. (2013) The ER stress transducer IRE1beta is required for airway epithelial mucin production. *Mucosal Immunol.* **6**, 639–654 [CrossRef PubMed](#)
- 22 Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K. (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107**, 881–891 [CrossRef PubMed](#)
- 23 Lee, A. H., Iwakoshi, N. N. and Glimcher, L. H. (2003) XBP1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol. Cell Biol.* **23**, 7448–7459 [CrossRef PubMed](#)
- 24 Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L. H. and Hotamisligil, G. S. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306**, 457–461 [CrossRef PubMed](#)
- 25 Harding, H. P., Zhang, Y. and Ron, D. (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* **397**, 271–274 [CrossRef PubMed](#)
- 26 Zinszner, H., Kuroda, M., Wang, X., Batchvarova, N., Lightfoot, R. T., Remotti, H., Stevens, J. L. and Ron, D. (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev.* **12**, 982–995 [CrossRef PubMed](#)
- 27 Marciniak, S. J., Yun, C. Y., Oyadomari, S., Novoa, I., Zhang, Y., Jungreis, R., Nagata, K., Harding, H. P. and Ron, D. (2004) CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.* **18**, 3066–3077 [CrossRef PubMed](#)
- 28 Hamanaka, R. B., Bobrovnikova-Marjon, E., Ji, X., Liebhaber, S. A. and Diehl, J. A. (2009) PERK-dependent regulation of IAP translation during ER stress. *Oncogene* **28**, 910–920 [CrossRef PubMed](#)
- 29 Brewer, J. W. (2013) Regulatory crosstalk within the mammalian unfolded protein response. *Cell. Mol. Life Sci.* **71**, 1067–1079 [CrossRef PubMed](#)
- 30 Hiramatsu, N., Messah, C., Han, J., Lavail, M. M., Kaufman, R. J. and Lin, J. H. (2014) Translational and posttranslational regulation of XIAP by eIF2alpha and ATF4 promotes ER stress-induced cell death during the unfolded protein response. *Mol. Biol. Cell* **25**, 1411–1420 [CrossRef PubMed](#)
- 31 Schindler, A. J. and Schekman, R. (2009) *In vitro* reconstitution of ER-stress induced ATF6 transport in COPII vesicles. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 17775–17780 [CrossRef PubMed](#)
- 32 Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M. and Mori, K. (2000) ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. *Mol. Cell Biol.* **20**, 6755–6767 [CrossRef PubMed](#)
- 33 Adachi, Y., Yamamoto, K., Okada, T., Yoshida, H., Harada, A. and Mori, K. (2008) ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum. *Cell Struct. Funct.* **33**, 75–89 [CrossRef PubMed](#)
- 34 Okada, T., Yoshida, H., Akazawa, R., Negishi, M. and Mori, K. (2002) Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. *Biochem. J.* **366**, 585–594 [CrossRef PubMed](#)
- 35 Surma, M. A., Klose, C., Peng, D., Shales, M., Mrejen, C., Stefanko, A., Braberg, H., Gordon, D. E., Vorkel, D., Ejsing, C. S. et al. (2013) A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress. *Mol. Cell* **51**, 519–530 [CrossRef PubMed](#)
- 36 Travers, K. J., Patil, C. K., Wodicka, L., Lockhart, D. J., Weissman, J. S. and Walter, P. (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* **101**, 249–258 [CrossRef PubMed](#)
- 37 Jesch, S. A., Zhao, X., Wells, M. T. and Henry, S. A. (2005) Genome-wide analysis reveals inositol, not choline, as the major effector of Ino2p-Ino4p and unfolded protein response target gene expression in yeast. *J. Biol. Chem.* **280**, 9106–9118 [CrossRef PubMed](#)
- 38 Jesch, S. A., Liu, P., Zhao, X., Wells, M. T. and Henry, S. A. (2006) Multiple endoplasmic reticulum-to-nucleus signaling pathways coordinate phospholipid metabolism with gene expression by distinct mechanisms. *J. Biol. Chem.* **281**, 24070–24083 [CrossRef PubMed](#)
- 39 Jonikas, M. C., Collins, S. R., Denic, V., Oh, E., Quan, E. M., Schmid, V., Weibezahn, J., Schwappach, B., Walter, P., Weissman, J. S. et al. (2009) Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. *Science* **323**, 1693–1697 [CrossRef PubMed](#)
- 40 Thibault, G., Shui, G., Kim, W., McAlister, G. C., Ismail, N., Gygi, S. P., Wenk, M. R. and Ng, D. T. (2012) The membrane stress response buffers lethal effects of lipid disequilibrium by reprogramming the protein homeostasis network. *Mol. Cell* **48**, 16–27 [CrossRef PubMed](#)
- 41 So, J. S., Hur, K. Y., Tarrilo, M., Ruda, V., Frank-Kamenetsky, M., Fitzgerald, K., Koteliensky, V., Lichtman, A. H., Iwakoshi, T., Glimcher, L. H. et al. (2012) Silencing of lipid metabolism genes through IRE1alpha-mediated mRNA decay lowers plasma lipids in mice. *Cell Metab.* **16**, 487–499 [CrossRef PubMed](#)
- 42 Fu, S., Yang, L., Li, P., Hofmann, O., Dicker, L., Hide, W., Lin, X., Watkins, S. M., Ivanov, A. R. and Hotamisligil, G. S. (2011) Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* **473**, 528–531 [CrossRef PubMed](#)
- 43 Cui, W., Ma, J., Wang, X., Yang, W., Zhang, J. and Ji, Q. (2013) Free fatty acid induces endoplasmic reticulum stress and apoptosis of beta-cells by Ca²⁺/calpain-2 pathways. *PLoS ONE* **8**, e59921 [CrossRef PubMed](#)
- 44 Promlek, T., Ishiwata-Kimata, Y., Shido, M., Sakuramoto, M., Kohno, K. and Kimata, Y. (2011) Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways. *Mol. Biol. Cell* **22**, 3520–3532 [CrossRef PubMed](#)
- 45 Volmer, R., van der Ploeg, K. and Ron, D. (2013) Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 4628–4633 [CrossRef PubMed](#)
- 46 Engel, A. and Gaub, H. E. (2008) Structure and mechanics of membrane proteins. *Annu. Rev. Biochem.* **77**, 127–148 [CrossRef PubMed](#)
- 47 Credle, J. J., Finer-Moore, J. S., Papa, F. R., Stroud, R. M. and Walter, P. (2005) On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18773–18784 [CrossRef PubMed](#)
- 48 Gupta, S., Deepti, A., Deegan, S., Lisbona, F., Hetz, C. and Samali, A. (2010) HSP72 protects cells from ER stress-induced apoptosis via enhancement of IRE1alpha-XBP1 signaling through a physical interaction. *PLoS Biol.* **8**, e1000410 [CrossRef PubMed](#)
- 49 Pincus, D., Chevalier, M. W., Aragon, T., van Anken, E., Vidal, S. E., El-Samad, H. and Walter, P. (2010) BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. *PLoS Biol.* **8**, e1000415 [CrossRef PubMed](#)

- 50 Dorner, A. J., Wasley, L. C. and Kaufman, R. J. (1992) Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. *EMBO J.* **11**, 1563–1571 [PubMed](#)
- 51 Okamura, K., Kimata, Y., Higashio, H., Tsuru, A. and Kohno, K. (2000) Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. *Biochem. Biophys. Res. Commun.* **279**, 445–450 [CrossRef PubMed](#)
- 52 Kimata, Y., Oikawa, D., Shimizu, Y., Ishiwata-Kimata, Y. and Kohno, K. (2004) A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1. *J. Cell Biol.* **167**, 445–456 [CrossRef PubMed](#)
- 53 Oikawa, D., Kimata, Y. and Kohno, K. (2007) Self-association and BiP dissociation are not sufficient for activation of the ER stress sensor Ire1. *J. Cell Sci.* **120**, 1681–1688 [CrossRef PubMed](#)
- 54 Gardner, B. M. and Walter, P. (2011) Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. *Science* **333**, 1891–1894 [CrossRef PubMed](#)
- 55 Kimata, Y., Ishiwata-Kimata, Y., Ito, T., Hirata, A., Suzuki, T., Oikawa, D., Takeuchi, M. and Kohno, K. (2007) Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. *J. Cell Biol.* **179**, 75–86 [CrossRef PubMed](#)
- 56 Welihinda, A. A., Tirasophon, W., Green, S. R. and Kaufman, R. J. (1997) Gene induction in response to unfolded protein in the endoplasmic reticulum is mediated through Ire1p kinase interaction with a transcriptional coactivator complex containing Ada5p. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4289–4294 [CrossRef PubMed](#)
- 57 Ron, D. and Walter, P. (2007) Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **8**, 519–529 [CrossRef PubMed](#)
- 58 Lee, K. P., Dey, M., Neculai, D., Cao, C., Dever, T. E. and Sicheri, F. (2008) Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. *Cell* **132**, 89–100 [CrossRef PubMed](#)
- 59 Squire, J., Zhou, A., Hassel, B. A., Nie, H. and Silverman, R. H. (1994) Localization of the interferon-induced, 2-5A-dependent RNase gene (RNS4) to human chromosome 1q25. *Genomics* **19**, 174–175 [CrossRef PubMed](#)
- 60 Zhou, A., Hassel, B. A. and Silverman, R. H. (1993) Expression cloning of 2-5A-dependent RNAase: a uniquely regulated mediator of interferon action. *Cell* **72**, 753–765 [CrossRef PubMed](#)
- 61 Korennykh, A. V., Egea, P. F., Korostelev, A. A., Finer-Moore, J., Zhang, C., Shokat, K. M., Stroud, R. M. and Walter, P. (2009) The unfolded protein response signals through high-order assembly of Ire1. *Nature* **457**, 687–693 [CrossRef PubMed](#)
- 62 Korennykh, A. V., Korostelev, A. A., Egea, P. F., Finer-Moore, J., Stroud, R. M., Zhang, C., Shokat, K. M. and Walter, P. (2011) Structural and functional basis for RNA cleavage by Ire1. *BMC Biol.* **9**, 47 [CrossRef PubMed](#)
- 63 Rubio, C., Pincus, D., Korennykh, A., Schuck, S., El-Samad, H. and Walter, P. (2011) Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity. *J. Cell Biol.* **193**, 171–184 [CrossRef PubMed](#)
- 64 Zhou, J., Liu, C. Y., Back, S. H., Clark, R. L., Peisach, D., Xu, Z. and Kaufman, R. J. (2006) The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 14343–14348 [CrossRef PubMed](#)
- 65 Ali, M. M., Bagratuni, T., Davenport, E. L., Nowak, P. R., Silva-Santisteban, M. C., Hardcastle, A., McAndrews, C., Rowlands, M. G., Morgan, G. J., Aherne, W. et al. (2011) Structure of the Ire1 autophosphorylation complex and implications for the unfolded protein response. *EMBO J.* **30**, 894–905 [CrossRef PubMed](#)
- 66 Cui, W., Li, J., Ron, D. and Sha, B. (2011) The structure of the PERK kinase domain suggests the mechanism for its activation. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 423–428 [CrossRef PubMed](#)
- 67 Dar, A. C., Dever, T. E. and Sicheri, F. (2005) Higher-order substrate recognition of eIF2alpha by the RNA-dependent protein kinase PKR. *Cell* **122**, 887–900 [CrossRef PubMed](#)
- 68 Hetz, C. (2012) The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.* **13**, 89–102 [PubMed](#)
- 69 Hetz, C., Chevet, E. and Harding, H. P. (2013) Targeting the unfolded protein response in disease. *Nat. Rev. Drug Discov.* **12**, 703–719 [CrossRef PubMed](#)
- 70 Gentile, C. L., Frye, M. and Pagliassotti, M. J. (2011) Endoplasmic reticulum stress and the unfolded protein response in nonalcoholic fatty liver disease. *Antioxid. Redox Signal.* **15**, 505–521 [CrossRef PubMed](#)
- 71 Rutkowski, D. T., Wu, J., Back, S. H., Callaghan, M. U., Ferris, S. P., Iqbal, J., Clark, R., Miao, H., Hassler, J. R., Fornek, J. et al. (2008) UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. *Dev. Cell* **15**, 829–840 [CrossRef PubMed](#)
- 72 Seo, J., Fortuno, E. S., 3rd, Suh, J. M., Stenesen, D., Tang, W., Parks, E. J., Adams, C. M., Townes, T. and Graff, J. M. (2009) Atf4 regulates obesity, glucose homeostasis, and energy expenditure. *Diabetes* **58**, 2565–2573 [CrossRef PubMed](#)
- 73 Kammoun, H. L., Chabanon, H., Hainault, I., Luquet, S., Magnan, C., Koike, T., Ferre, P. and Foufelle, F. (2009) GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. *J. Clin. Invest.* **119**, 1201–1215 [CrossRef PubMed](#)
- 74 Pineau, L., Colas, J., Dupont, S., Beney, L., Fleurat-Lessard, P., Berjeaud, J. M., Berges, T. and Ferreira, T. (2009) Lipid-induced ER stress: synergistic effects of sterols and saturated fatty acids. *Traffic* **10**, 673–690 [CrossRef PubMed](#)
- 75 Mayer, C. M. and Belsham, D. D. (2010) Palmitate attenuates insulin signaling and induces endoplasmic reticulum stress and apoptosis in hypothalamic neurons: rescue of resistance and apoptosis through adenosine 5' monophosphate-activated protein kinase activation. *Endocrinology* **151**, 576–585 [CrossRef PubMed](#)
- 76 Park, S. W. and Ozcan, U. (2013) Potential for therapeutic manipulation of the UPR in disease. *Semin. Immunopathol.* **35**, 351–373 [CrossRef PubMed](#)
- 77 Park, S. W., Zhou, Y., Lee, J., Lu, A., Sun, C., Chung, J., Ueki, K. and Ozcan, U. (2010) The regulatory subunits of PI3K, p85alpha and p85beta, interact with XBP-1 and increase its nuclear translocation. *Nat. Med.* **16**, 429–437 [CrossRef PubMed](#)
- 78 Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C. M. and Stefani, M. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* **416**, 507–511 [CrossRef PubMed](#)
- 79 Taylor, J. P., Hardy, J. and Fischbeck, K. H. (2002) Toxic proteins in neurodegenerative disease. *Science* **296**, 1991–1995 [CrossRef PubMed](#)
- 80 Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoishi, S., Yokochi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**, 605–608 [CrossRef PubMed](#)
- 81 Silva, R. M., Ries, V., Oo, T. F., Yarygina, O., Jackson-Lewis, V., Ryu, E. J., Lu, P. D., Marciniak, S. J., Ron, D., Przedborski, S. et al. (2005) CHOP/GADD153 is a mediator of apoptotic death in substantia nigra dopamine neurons in an *in vivo* neurotoxin model of parkinsonism. *J. Neurochem.* **95**, 974–986 [CrossRef PubMed](#)



- 82 MacDonald, M. E., Ambrose, C. M., Duyao, M. P., Myers, R. H., Lin, C., Srinidhi, L., Barnes, G., Taylor, S. A., James, M., Groot, N. et al. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* **72**, 971–983 [CrossRef PubMed](#)
- 83 Hipp, M. S., Patel, C. N., Bersuker, K., Riley, B. E., Kaiser, S. E., Shaler, T. A., Brandeis, M. and Kopito, R. R. (2012) Indirect inhibition of 26S proteasome activity in a cellular model of Huntington's disease. *J. Cell Biol.* **196**, 573–587 [CrossRef PubMed](#)
- 84 Carnemolla, A., Fossale, E., Agostoni, E., Michelazzi, S., Calligaris, R., De Maso, L., Del Sal, G., MacDonald, M. E. and Persichetti, F. (2009) Rrs1 is involved in endoplasmic reticulum stress response in Huntington disease. *J. Biol. Chem.* **284**, 18167–18173 [CrossRef PubMed](#)
- 85 Varshney, A. and Ehrlich, B. E. (2003) Intracellular Ca²⁺ signaling and human disease: the hunt begins with Huntington's. *Neuron* **39**, 195–197 [CrossRef PubMed](#)
- 86 Hardy, J. and Allsop, D. (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci.* **12**, 383–388 [CrossRef PubMed](#)
- 87 Hoozemans, J. J., Veerhuis, R., Van Haastert, E. S., Rozemuller, J. M., Baas, F., Eikelenboom, P and Scheper, W. (2005) The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol.* **110**, 165–172 [CrossRef PubMed](#)
- 88 Hoozemans, J. J., van Haastert, E. S., Nijholt, D. A., Rozemuller, A. J., Eikelenboom, P and Scheper, W. (2009) The unfolded protein response is activated in pretangle neurons in Alzheimer's disease hippocampus. *Am. J. Pathol.* **174**, 1241–1251 [CrossRef PubMed](#)
- 89 Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K. et al. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754–760 [CrossRef PubMed](#)
- 90 Queitsch, C., Sangster, T. A. and Lindquist, S. (2002) Hsp90 as a capacitor of phenotypic variation. *Nature* **417**, 618–624 [CrossRef PubMed](#)
- 91 Little, J. L., Wheeler, F. B., Fels, D. R., Koumenis, C. and Kridel, S. J. (2007) Inhibition of fatty acid synthase induces endoplasmic reticulum stress in tumor cells. *Cancer Res.* **67**, 1262–1269 [CrossRef PubMed](#)
- 92 Vandewynckel, Y. P., Laukens, D., Geerts, A., Bogaerts, E., Paridaens, A., Verhelst, X., Janssens, S., Heindryckx, F. and Van Vlierberghe, H. (2013) The paradox of the unfolded protein response in cancer. *Anticancer Res.* **33**, 4683–4694 [PubMed](#)
- 93 Bi, M., Naczki, C., Koritzinsky, M., Fels, D., Blais, J., Hu, N., Harding, H., Novoa, I., Varia, M., Raleigh, J. et al. (2005) ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *EMBO J.* **24**, 3470–3481 [CrossRef PubMed](#)
- 94 Dong, D., Ni, M., Li, J., Xiong, S., Ye, W., Virrey, J. J., Mao, C., Ye, R., Wang, M., Pen, L. et al. (2008) Critical role of the stress chaperone GRP78/BiP in tumor proliferation, survival, and tumor angiogenesis in transgene-induced mammary tumor development. *Cancer Res.* **68**, 498–505 [CrossRef PubMed](#)
- 95 Friedman, A. D. (1996) GADD153/CHOP, a DNA damage-inducible protein, reduced CAAT/enhancer binding protein activities and increased apoptosis in 32D c13 myeloid cells. *Cancer Res.* **56**, 3250–3256 [PubMed](#)
- 96 Li, G., Mongillo, M., Chin, K. T., Harding, H., Ron, D., Marks, A. R. and Tabas, I. (2009) Role of ERO1-alpha-mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. *J. Cell Biol.* **186**, 783–792 [CrossRef PubMed](#)
- 97 Ozcan, U., Ozcan, L., Yilmaz, E., Duvel, K., Sahin, M., Manning, B. D. and Hotamisligil, G. S. (2008) Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. *Mol. Cell* **29**, 541–551 [CrossRef PubMed](#)

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