Lipid bilayer stress and proteotoxic stress-induced unfolded protein response deploy divergent transcriptional and non-transcriptional programmes

Fun, Xiu Hui; Thibault, Guillaume

2020

Fun, X. H. & Thibault, G. (2020). Lipid bilayer stress and proteotoxic stress-induced unfolded protein response deploy divergent transcriptional and non-transcriptional programmes. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids, 1865(1), 1-9. doi:10.1016/j.bbalip.2019.04.009

https://hdl.handle.net/10356/136602

https://doi.org/10.1016/j.bbalip.2019.04.009; https://doi.org/212240; https://doi.org/212240

Lipid bilayer stress and proteotoxic stress-induced unfolded protein response deploy divergent transcriptional and non-transcriptional programmes

Xiu Hui Fun and Guillaume Thibault*

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

ORCID
XHF 0000-0002-5371-0394
GT 0000-0002-7926-4812

*Corresponding author:
Guillaume Thibault
School of Biological Sciences
Nanyang Technological University
Singapore 637551
email: thibault@ntu.edu.sg
Phone: +65 6592 1787

ABSTRACT

The unfolded protein response (UPR) is activated by endoplasmic reticulum (ER) stress and is designed to restore cellular homeostasis through multiple intracellular signalling pathways. In mammals, the UPR programme regulates the expression of hundreds of genes in response to signalling from ATF6, IRE1, and PERK. These three highly conserved stress sensors are activated by the accumulation of unfolded proteins within the ER. Alternatively, IRE1 and PERK sense generalised lipid bilayer stress (LBS) at the ER while ATF6 is activated by an increase of specific sphingolipids. As a result, the UPR supports cellular robustness as a broad-spectrum compensatory pathway that is achieved by deploying a tailored transcriptional programme adapted to the source of ER stress. This review summarises the current understanding of the three ER stress transducers in sensing proteotoxic stress and LBS. The plasticity of the UPR programme in the context of different sources of ER stress will also be discussed.

KEYWORDS

Endoplasmic reticulum stress, unfolded protein response, lipid bilayer stress, proteotoxic stress, stress sensing mechanism, differential transcriptome.

HIGHLIGHTS

- ER stress sensors activate the UPR by two independent sensing mechanisms
- Non-specific lipid stress actives IRE1 and PERK while ATF6 responds to sphingolipid
- The deployed transcriptional UPR programme is adapted to the source of ER stress

ABBREVIATIONS

COPII, coat protein II; DHC, dihydroceramide; DHS, dihydrosphingosine; ER, endoplasmic reticulum; ERAD,

66 ER-associated protein degradation; ERSE, ER stress response element; LBS, lipid bilayer stress; LDL, low-
67 density lipoprotein; NAFLD, nonalcoholic fatty liver disease; RIDD, IRE1-dependent decay; SRP, signal
68 recognition particle; UPR, unfolded protein response; UPR^{LBS}, lipid bilayer stress-induced UPR; UPR^{PT},
69 proteotoxic-induced UPR; UPRE, UPR element.

1. INTRODUCTION

The unfolded protein response (UPR) is a eukaryotic stress response pathway canonically activated by endoplasmic reticulum (ER) stress. Equipped with different mechanisms including translational attenuation [1] and transcriptional activation of genes involved in ER protein folding and degradation [2, 3], the activation of the UPR is required to overcome ER stress. The UPR is conserved from protozoa but exhibits a high degree of complexity in metazoans [4]. The mammalian UPR programme regulates the expression of hundreds of genes in response to signalling from the three sensor molecules ATF6 (activating transcription factor 6), IRE1 (inositol requiring enzyme 1), and PERK (double-stranded RNA-activated protein kinase (PKR)–like ER kinase), all three of which are embedded within the ER membrane (Fig. 1). IRE1 is the most evolutionarily conserved UPR sensor, and it is the sole sensor present in lower eukaryotes including *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. As part of the initial adaptive phase of the UPR, accumulation of unfolded proteins within the ER lumen activates the three ER stress sensors which further initiates a cascade of signalling events that aim to restore and protect cellular integrity [5, 6]. However, cellular dysfunction and cell death often occurs when ER stress is chronically prolonged and protein load overwhelms the folding capacity of the ER. Additionally, the three transducer proteins sense ER stress through lipid bilayer stress (LBS), in a manner independent from their respective luminal domains [7-11]. The molecular mechanism of IRE1 activation upon LBS has been recently demonstrated in budding yeast [7]. Various perturbations of lipid metabolism have been shown to activate IRE1, including altered ratio of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) [10], elevated lipid saturation [12], elevated sterol levels [13], and inositol depletion [9]. Hence, LBS was introduced as a collective term for atypical lipid compositions of the ER membrane that can contribute to UPR activation [12].

The transcriptional UPR programme must directly address specific defective or saturated pathways while simultaneously avoiding overcompensating for others that are functional as a constitutively activated UPR in unstressed WT yeast cells is toxic [14]. It can be hypothesised that the UPR transitions from a general response towards a curated transcriptional programme to address the source of stress through a negative feedback loop mechanism. Initially, the UPR transducers are either activated by LBS, proteotoxic stress, or both resulting in temporary translational attenuation and the upregulation of genes. Through the responsive phase, different UPR-related players modify the initial transcriptional programme. Depending on the cell type and organism, these factors are divergent, and their activity might be influenced by the source of stress. Thus, the UPR transcriptional programme displays plasticity according to the specific needs of the cell to restore ER homeostasis. This review will provide an overview on how the different ER stress transducers sense LBS and proteotoxic stress independently, before discussing the transcriptional and non-transcriptional UPR programmes that are associated with proteotoxic-induced ER stress (UPR^{PT}) and LBS-induced ER stress (UPR^{LBS}).

2. ER stress transducers sense lipid bilayer stress and proteotoxic stress by different mechanisms

In metazoans, activation of the UPR involves three ER localized sensors IRE1, PERK and ATF6 that sense and employ unique transduction mechanisms to the cytosol and nucleus to enable an adaptive response. Each unique sensor consists of an ER luminal domain that senses aberrant conditions within the ER lumen and/or membrane compositions, an ER-transmembrane domain, and a cytosolic domain that relay information to the transcriptional and translational machineries. First identified in *S. cerevisiae*, the UPR transducer Ire1 is the most evolutionarily conserved ER stress sensor [15, 16]. Ire1 dimerises and further clusters in higher-ordered oligomers upon UPR^{LBS} and UPR^{PT} [7, 17, 18]. Clustering of Ire1 leads to transphosphorylation and the

activation of its unique ribonuclease domain. Subsequently, active Ire1 cleaves the intron of homologous to Atf/Creb1 (*HAC1*) mRNA, which is translated into the transcription factor Hac1 that upregulates the expression of hundreds of genes involved in various cellular processes to restore ER homeostasis [10, 19, 20]. In mammals, IRE1α and IRE1β are the two IRE1 homologues of yeast Ire1 [21, 22], where IRE1α is ubiquitously expressed and therefore the main UPR mediator, while IRE1β expression is limited to the epithelial cells of the gastrointestinal tract [22, 23]. Conserved in higher organisms, IRE1α is similarly activated and it splices the pre-mRNA of the transcription factor X-box binding protein 1 (*XBP1*) [24, 25] (Fig. 1A). In addition to the interaction between IRE1α and the Sec61 translocon complex, the signal recognition particle (SRP) pathway recruitment of $XBP1^{u}$ to the Sec61 translocon is also required for efficient cleavage of $XBP1^{u}$ [26, 27]. The spliced *XBP1* mRNA is then translated into an active transcription factor that induces UPR target genes to alleviate ER stress. In the event of prolonged ER stress, IRE1α cleaves mRNAs localised to the ER membrane by a mechanism termed as regulated IRE1-dependent decay of mRNA (RIDD) (Fig. 1A) [28, 29]. The activation of RIDD further complements the UPR by reducing the incoming protein load on the ER under stress conditions. The addition of two new classes of ER stress sensors, PERK and ATF6, has increased the complexity of the ER stress sensing and response mechanisms in higher eukaryotes. Oligomerisation of PERK triggers autophosphorylation and the phosphorylation of eukaryotic translation initiation factor 2 subunit alpha (eiF2α) during UPR^{LBS} and UPR^{PT} [11] (Fig. 1B). Phosphorylated eiF2α (eIF2α-P) attenuates general protein translation while enhancing the translation of the activating transcription factor 4 (ATF4). Subsequently, ATF4 induces a subset of UPR genes including pro-apoptotic factor C/EBP-homologous protein (CHOP) and the growth arrest and DNA damage-inducible protein (GADD34). GADD34 promotes the dephosphorylation of eIF2α-P to subsequently derepress translation. The transcription factor CHOP induces pro-apoptotic genes in the presence of persistent stress such as prolonged ER stress [30]. In contrast to IRE1α and PERK, disulfide-

bonded ATF6 is reduced during ER stress and the resulting monomeric form is transported to the Golgi apparatus through interaction with the coat protein II (COPII) [31, 32] (Fig. 1C). In the Golgi, the luminal and the transmembrane domains of ATF6 are cleaved by site-1 and site-2 proteases (S1P and S2P) which releases the cytosolic N-terminal domain of ATF6 [ATF6(N)]. In turn, ATF6(N) is transported to the nucleus where it upregulates a subset of UPR genes. In parallel, many organisms have a general homeostatic response to limit protein load in the ER by upregulating genes that increase the ER folding capacity, attenuating translation, and degrading ER-targeted mRNAs. Together, the activation of these three sensors aids in protecting against proteotoxicity and to maintain overall ER homeostasis.

2.1. Sensing proteotoxic stress at the ER

Protein glycosylation is a sophisticated post-translational modification that plays an important role in the folding, stability, subcellular localisation and biological function of proteins residing or transiting through the secretory pathway. Initially known to function as a natural antibiotic, tunicamycin inhibits *N*-linked glycosylation in the ER of eukaryotic cells, thereby leading to the disruption of protein folding and the severe accumulation of unfolded proteins within the ER [33-35]. Pioneer work to dissect the ER stress sensing mechanism pointed to the accumulation of unfolded proteins in the ER lumen. In the late 80's, either the expression of unfolded influenza haemagglutinin viral protein or tunicamycin treatment were shown to induce the expression of the ER-resident molecular chaperones immunoglobulin heavy-chain-binding protein (BiP), 94 kDa glucose-regulated protein (GRP94), and protein disulfide-isomerase (PDI) [36, 37]. ER molecular chaperones facilitate protein translocation, folding, and quality control [20]. Subsequently, the missing link between ER stress and gene regulation, including ER-resident molecular chaperones, was elucidated in *S. cerevisiae*. The accumulation of unfolded proteins was found to induce the splicing of *HAC1* mRNA in an Ire1-dependent manner, resulting in

158 the translation of the transcription factor Hac1 [15, 38, 39]. The IRE1α/XBP1 axis was eventually identified in
159 mammals and to be similar to the yeast Ire1/Hac1 axis [21, 22, 25]. During the same era, inhibition of protein
160 synthesis was reported to correlate with the phosphorylation of eIF2α in response to ER stress signalling in
161 mammals [40-42] (Fig. 1). Later, PERK and ATF6 were reported to be activated upon the accumulation of
162 unfolded proteins [1, 43, 44]. Together, the three ER stress transducers were thought to be solely activated by
163 the accumulation of unfolded proteins in a similar fashion while having their unique properties and activation
164 pathways.
165
166 Our current understanding on how the ER stress transducer IRE1 senses the accumulation of unfolded
167 proteins at the ER is mostly derived from structural and *in vivo* studies in yeast. Despite early discovery on the
168 role of Ire1 as a central UPR regulator, the mechanism by which it senses unfolded proteins remains disputed.
169 There are two alternative models used to describe how Ire1's luminal domain sense ER stress: an earlier
170 model where Ire1 activity is regulated by the release of the molecular chaperone Kar2 (BiP homologue) and a
171 recent model where unfolded proteins act as activating ligands that directly binds Ire1. In the first model, BiP
172 keeps Ire1 in its inactive monomeric states through the binding of their luminal domains under normal
173 conditions. In cells undergoing ER stress, BiP binding moves from Ire1 to the then accumulating unfolded
174 proteins, allowing Ire1 to oligomerise and to activate the downstream pathways. Supporting this model,
175 immunoprecipitation assays showed that Ire1 remains associated to BiP in unstressed cells and dissociates
176 from BiP under ER stress conditions [45-47]. Consistently, overexpression of BiP in CHO cells showed a
177 greater amount of BiP associated with PERK or IRE1 than in parental CHO cells with endogenous BiP levels
178 [45]. Additionally, in BiP overexpressing CHO cells, ER stress-induced phosphorylation of PERK was delayed
179 and incomplete while IRE1 activation was absent [45-47]. Together, the emerging model pointed to the role of

Kar2/BiP in keeping IRE1 as an inactive monomer under non-stress condition. Upon unfolded protein accumulation, Kar2/BiP is titrated away from monomeric IRE1 to bind unfolded proteins. Consequently, monomeric IRE1 molecules, freed of Kar2/BiP, oligomerises and thus self-activate.

In contrast to the competitive model, where IRE1 competes with misfolded proteins to bind BiP, a recent *in vitro* study supports an allosteric model in which the interaction between IRE1 and BiP occurs through the ATPase domain of BiP, independently of nucleotides [48]. This is further supported by an *in vitro* Forster resonance energy transfer (FRET) UPR induction assay that revealed the ATPase domain of BiP as being essential for the interaction with the luminal domain of IRE1 [49]. As the binding between IRE1, BiP and unfolded proteins involves two different domains of BiP and is coupled to its conformation change, this is not considered as a competitive process, but rather an allosteric model for UPR activation upon ER stress [49]. However, Ire1 mutants lacking the juxtamembrane segment of its luminal domain that is responsible for BiP binding maintained their ability to get activated upon ER stress and remains inactive in its absence, suggesting that the release and rebinding of BiP is not the principal determinant for the activation of Ire1 [17, 47, 50, 51]. Instead, the activation of Ire1 could be a hybrid model in which the first step involves the release of BiP, followed by the binding of unfolded proteins to the luminal domain [17, 47]. Another alternative model of Ire1 regulation involves the direct binding of unfolded proteins to its luminal domain, triggering self-oligomerisation and activation of the cytoplasmic effector domains of Ire1. This model is supported by structural studies of Ire1 luminal domain that revealed a putative peptide binding groove that resembles the peptide binding domains of major histocompatibility complexes (MHCs) [17, 47]. Further mutational analysis showed that residues facing the groove are essential for UPR activation and their mutations reduce the response [50]. In addition, *in vivo* and *in vitro* studies showed that yeast Ire1 directly interacts with misfolded mutant of carboxypeptidase Y

202 (CPY*) and peptides, respectively, leading to its oligomerisation [52]. Despite the structural differences
203 between human and yeast IRE1 luminal domains, the crystal structure of human IRE1α core luminal domain
204 displays conserved structural elements in its core [53]. As in yeast, peptides and unfolded proteins bind to the
205 MHC-like groove within the luminal domain of IRE1α, inducing conformational changes that result in its
206 oligomerisation [53]. Impairment of the oligomerisation of the core luminal domain of IRE1 abrogated its activity
207 in living cells, further indicating that IRE1 activation is dependent on unfolded protein binding-induced
208 oligomerisation [53]. Together, these observations demonstrated that the direct binding of unfolded proteins to
209 the luminal domain of IRE1 induces its oligomerisation and further leads to the UPR activation (Fig. 2A).
210
211 BiP also binds the luminal domain of PERK, negatively regulating PERK oligomerisation and
212 autophosphorylation [45, 48, 54]. Although it has been well established that dimer formation is adequate for
213 autophosphorylation for both PERK [54] and Ire1 [16, 55-58], structural and functional analyses revealed the
214 transient formation of a novel tetrameric conformation of PERK that possess high efficiency of
215 autophosphorylation [59]. The transition between PERK dimer and tetramer may be an indication of an intrinsic
216 form of regulation that is dependent on the level of unfolded proteins within the ER lumen. Further biochemical
217 and structural findings showed that the oligomerisation of PERK luminal domains is dependent on the direct
218 binding of unfolded proteins to activate the UPR [60]. PERK luminal domain contains a highly flexible peptide-
219 binding grove which recognises and interacts with a variety of unfolded proteins. Flexible binding regions have
220 been reported for several molecular chaperones [61-66], hence the peptide binding groove of PERK possibly
221 varies according to the size of its peptide ligand to bind a wide range of unfolded proteins.
222
223 ATF6 is a type II transmembrane protein with a carboxyl-terminal luminal domain and an amino-terminal basic

leucine zipper (bZIP) transcription factor domain [43, 44]. The luminal domain of ATF6 bears no sequence homology to either IRE1 or PERK, and its mechanism of activation is dependent on changes in subcellular localization and intramembrane proteolysis [43, 67]. The luminal domain of ATF6 is essential and sufficient for its activation under ER-stressed conditions, indicating that this domain senses ER stress signal within the ER [68]. Subsequently, BiP has been shown to bind to the luminal domain of ATF6 and to regulate its activation [69]. Binding of BiP retains ATF6 in the ER by hindering its Golgi localization signals (GLSs) while the dissociation of BiP during ER stress promotes the transportation of ATF6 to the Golgi [69]. Despite sharing a similar mode of regulation by BiP with IRE1 and PERK, a mutational study showed that BiP-ATF6 complex is stable and BiP dissociation during ER stress occurs through an active mechanism rather than the competition with unfolded proteins described above for IRE1 [70]. Unlike IRE1 and PERK, ATF6 exists as a monomer, dimer and oligomer, with the higher order forms maintained during non-ER-stressed condition via inter- and intramolecular disulfide bridges formed between the two conserved cysteine residues within its luminal domain [32]. Mutation of both conserved cysteine residues that mediate oligomerisation failed to induce constitutive trafficking of ATF6 to the Golgi apparatus, suggesting that monomeric ATF6 is not sufficient for its activation [32]. Upon ER stress, a reduced form of monomeric ATF6 translocates to the Golgi apparatus where its amino-terminal transcription factor domain is released by S1P and S2P-mediated proteolysis. The conversion to the monomeric form of ATF6 seems to be important for its activation as oligomers are absent in the Golgi apparatus and they are also poor substrates of S1P cleavage [32]. The reduction process is specific to ATF6, as another ER membrane-bound transcription factor, LZIP, was not reduced upon ER stress [32]. In addition, the glycosylation inhibitor tunicamycin reduced ATF6 similar to the reducing agent DTT, reflecting the correlation between reduction and ATF6 activation [32]. This further suggests that the mechanism underlying the activation of ATF6 seems to be rather strict and is dependent on changes within the ER [32, 71]. Since BiP dissociation

246 and reduction of disulphide bonds are closely associated with the luminal domain of ATF6, identification of
247 oxidoreductases modulating ER stress-induced reduction of ATF6 will contribute to a better understanding of
248 ATF6-mediated ER stress sensing mechanism [72].
249
250 *2.2. Sensing lipid bilayer stress at the ER*
251 The UPR programme upregulates a subset of lipid synthesis genes, indicative of interdependence between
252 lipids and ER stress [19]. Accordingly, several studies have reported on the link between the disruption of lipid
253 homeostasis and the activation of the UPR in eukaryotes [8, 10, 73-76]. One of the earliest observations that
254 obesity-induced UPR activation involves ER stress came from studies utilizing chemical chaperones [77].
255 These chemical chaperones stabilise protein conformation, improve ER folding capacity, facilitate appropriate
256 trafficking of mutant proteins and act as buffer in the presence of unfolded proteins, linking lipid-induced ER
257 stress to the accumulation of unfolded proteins [13, 77-82]. However, several groups have subsequently
258 demonstrated that the three ER stress sensors are activated by LBS independently of unfolded protein
259 accumulation [8, 9, 11, 83].
260
261 In yeast, Ire1 lacking its luminal stress-sensing domain (Ire1ΔLD) that binds unfolded proteins, when dimerised,
262 was shown to activate the UPR during LBS but failed to do so with the overexpression of CPY* [9]. In the study,
263 LBS was induced by either the depletion of the phosphatidylinositol and sphingolipids precursor inositol from
264 the growth medium or genetic perturbation of the lipid biosynthesis pathways [9]. The study suggested that
265 Ire1 contains distinct ER stress sensors, one in its luminal domain and the other within the transmembrane or
266 cytosolic domains [9]. Similar findings were reported in mammalian cells for both IRE1α and PERK where LBS
267 was induced by abnormally elevated levels of saturated fatty acids within the biological membrane [11]. IRE1α

and PERK, lacking their luminal stress-sensing domain (IRE1αΔLD and PERKΔLD, respectively), were sufficient to sense LBS but were insensitive to proteotoxic stress. These findings showed that LBS can be sensed by the transmembrane domains of IRE1α and PERK. Due to the decrease in membrane fluidity through fatty acid saturation, they proposed that the transmembrane domains of IRE1α and PERK are favourably excluded from the gel phase, thus favouring their oligomerisation. Despite swapping the transmembrane domain of an unrelated ER protein and sequence scrambling, both IRE1 and PERK still retained their ability to activate the UPR. This suggests that although responsiveness to lipid perturbation requires the presence of a transmembrane domain, it is not sequence specific [11, 84]. A conserved amphipathic helix within the luminal domain of Ire1, in proximity to the transmembrane domain, was shown to be necessary to drive the oligomerisation of Ire1 during LBS in yeast [7]. The unique properties of the amphipathic helix together with the transmembrane domain of Ire1 was proposed to drive local membrane compression and acyl chain disordering which favour the oligomerisation of Ire1 during LBS. Together, the conserved LBS sensing mechanism by IRE1 and PERK appears to be dependent on their secondary structures at the membrane and the surrounding membrane biophysical properties rather than specific amino acid sequences (Fig. 2B).

Recently, ATF6 was reported to be specifically activated from LBS but through a divergent sensing mechanism from those of IRE1α and PERK [83]. ATF6 was shown to be sensitive to the accumulation of two sphingolipid species, namely dihydrosphingosine (DHS) and dihydroceramide (DHC), while being non-responsive to ceramides (Fig. 2C). Although both sphingolipid intermediates are normally in low abundance, the authors demonstrated their levels are elevated upon ER stress. DHS and DHC accumulation activate ATF6 similarly as proteotoxic stress (Fig. 1C). Single point mutations within the ATF6 transmembrane conserved motif were sufficient to prevent sphingolipid-induced activation whereas it remained sensitive to proteotoxic stress.

290 Interestingly, the ATF6 transmembrane conserved VXXFIXXNY motif that is similar to the sphingomyelin
291 binding motif of p24, a major transmembrane protein of COPI-coated transport vesicles [85]. The possibility of
292 other types of LBS in activating ATF6 should be explored. Whether LBS disrupts COPII-mediated ATF6
293 transport to the Golgi apparatus or attenuates S1P/S2P enzymatic activities should also be investigated.
294
295 In addition to the UPR sensors, other ER-resident proteins sense specific lipid species to maintain lipid
296 homeostasis. To maintain membrane fluidity, fatty acid saturation is tightly regulated to ensure cell functionality
297 and vitality. For instance in yeast, the lack of the Δ9-fatty acid desaturase *OLE1* results in lethality which can
298 be rescued with unsaturated fatty acids supplementation [86, 87]. The expression level of *OLE1* is controlled
299 by the ER membrane-bound transcriptional regulator Mga2 [88-90]. The oligomeric transmembrane α-helix of
300 yeast Mga2 undergoes subsequent rotational orientations that induce conformational changes upon alteration
301 of the fatty acid composition within the membrane and is activated upon proteolytic cleavage. Active Mga2
302 further adjusts *OLE1* expression to maintain membrane integrity and cell viability [91]. Cholesterol synthesis
303 is regulated at the ER by the membrane-bound transcription factor sterol regulatory element binding element
304 (SREBP) [92, 93]. The activation of SREBP employs the same cellular machinery as ATF6 as it requires
305 transport to the Golgi apparatus followed by sequential cleavage by the S1P/S2P to release its soluble
306 transcription factor when cholesterol is low at the ER [94]. The release of soluble SREBP further induces the
307 expression of cholesterol synthesis enzymes, triglycerides, phospholipids and the low-density lipoprotein (LDL)
308 receptor [95]. Additionally, sterol synthesis is regulated at the translational level through the HMG-CoA
309 reductase (HMGR) protein. The accumulation of intermediate products through the cholesterol biosynthesis
310 pathway promotes the degradation of HMGR [96], consequently preventing excessive accumulation of sterol.
311 Together, these lipid sensors contribute to lipid homeostasis at the ER but independently of the UPR pathway.

3. The deployed UPR transcriptional and non-transcriptional programme is reflective to the source of ER stress

Equipped with different mechanisms including translational attenuation [1] and transcriptional activation of genes involved in ER protein folding and degradation [2, 3], the UPR broadly shifts the transcriptome to overcome ER stress (Fig. 3). The expression levels of the UPR transducers differ across cell types, providing plasticity to a wide variety of ER-stress stimulus [97] (Fig. 3). For instance, ER molecular chaperones and the majority of UPR target genes display tissue-specific effects that is XBP-1-dependent in plasma B cells and fibroblasts but are not affected by the deletion of *Xbp1* in pancreatic cells [98]. Additionally, both genome-wide and computational analyses revealed that XBP1 regulates tissue- and condition-specific target genes in skeletal muscle and secretory cells [99]. The UPR transcriptional programme displays greater flexibility where an extensive range of inputs can activate various overlapping but specific pathways that result in differential outputs according to cellular needs.

Stress responses usually act within a cell experiencing unfavourable conditions that in turn promote proteotoxicity. However, diseases and cellular damages that involve unfolded proteins are hardly confined within a single tissue. Hence, the mechanisms that protect or promote disease progression require communication between cells and tissues, namely cell-nonautonomous regulatory pathways [100] (Fig. 3). In addition to the conventional intracellular ER-to-nucleus signal transduction pathway, the UPR can be activated extrinsically through factors released from cancer cells [101]. Similar UPR-activated cell-nonautonomous communication occurs where neuronal cells constitutively activated UPR induces the UPR of intestinal cells, resulting in *C. elegans* lifespan extension [102, 103].

The ER stress-induced transcriptional UPR programmes are well defined. For instance, transcriptional profiling experiments identified ~381 UPR target genes that are induced upon DTT and tunicamycin treatment in budding yeast [19]. As expected, expression of ER chaperones was upregulated, together with the expression of genes involved in various functions including protein trafficking and quality control, lipid metabolism and cell wall biosynthesis [19]. Additionally, the three UPR transducers were shown to upregulate inducible UPR genes with similar functions upon tunicamycin treatment in *C. elegans*, with *ire-1*/*xbp-1* and *pek-1* as major contributors [104]. The ability of the UPR to regulate a wide variety of genes was further supported by a systematic analysis of 4,500 yeast deletion mutants that identified ~10% mutant strains with a significant UPR activation [105]. Therefore, it is not surprising that ER stress induced by proteotoxic or LBS might differentially alter the UPR to reach cellular homeostasis. UPR^{LBS} and UPR^{PT} were shown to be distinct from each other in which the UPR activation in both yeast and *C. elegans* are upregulated independently of unfolded protein levels [106, 107]. In yeast, we have demonstrated that the UPR elicits a broad response that remodels the proteome to protect against conditions that alters lipid composition of membranes using global transcriptional and proteomic studies [10]. Similarly, we reported a different UPR transcriptional programme that was deployed upon LBS in comparison to proteotoxic stress in yeast and *C. elegans* [8, 10]. Despite these differences, a subset of genes in *C. elegans* were found to be similarly regulated by both UPR^{PT} and UPR^{LBS}, suggesting a shared programme regardless of the source of ER stress [8]. Consistent with previous findings, there are several processes that are similarly regulated including protein quality control, lipid metabolism, vesicle trafficking, and protein synthesis [8-10, 19, 104]. Among the UPR-upregulated genes, a subset of genes is well characterised while a large proportion of these genes and their roles to restore ER homeostasis remains unexplored. Therefore, the minimal UPR transcriptional programme necessary to restore ER homeostasis is

unknown. Better understanding the slight variations of the transcriptional programme is critical to develop new successful therapies. UPR inhibitors that have been developed to treat diseases have serious potential long-term side effects on the functions of the pancreas, the immune system, and the liver as the UPR transcriptional programme is too broad to be inhibited from the upstream players [108]. As the UPR is either pro-survival or pro-apoptotic, the UPR transcriptional programme can be potentially manipulated to ease ER stress by promoting apoptosis [108]. Thus, inhibiting a subset of downstream UPR target genes could yield better therapeutic success, but the characterisation of such players, in different stress conditions remain to be identified.

3.1. Transcriptional and non-transcriptional UPR programme upon proteotoxic stress

Each UPR transducer regulates its downstream transcription factor, and the combination of the three UPR branches generates a variety of responses. These transcription factors also regulate an overlapping subset of genes involved in both ER-specific and general cellular proteostasis pathways [109-112]. Among the hundreds of upregulated genes in eukaryotes, a subset encodes ER chaperones, protein trafficking machinery, protein quality control, lipid metabolism, and autophagy [8, 19, 20, 24, 25, 113]. These genes are rapidly and efficiently transcribed by the presence of ER stress response element (ERSE) and UPR element (UPRE) within their promoter regions. To increase specificity, certain genes contain unique self-regulatory loops to further promote the robustness of the UPR in response to different cellular stresses [114]. For instance, transcriptional levels of ERSE-bearing genes *GRP78* (BiP protein), *XBP1*, and *CHOP* are increased by thapsigargin-induced depletion of ER stored calcium, which can be further self-amplified with higher concentration of thapsigargin. On the other hand, UPRE-bearing genes ATF4, ATF6, IRE1, and PERK lack positive feedback loops upon acute ER stress. In addition to temporary translation attenuation through eIF2α phosphorylation, PERK

activates the nuclear factor-erythroid 2-related factor 2 (NRF2) to upregulate genes encoding antioxidant proteins and detoxifying enzymes [115]. Both PERK and IRE1 independently induce the transcription factor NF-κB to potentially promote inflammation, immune responses, apoptosis and tumorigenesis [116, 117] (Fig. 1A).

ATF4-induced CHOP regulates the growth arrest and GADD34, a regulatory subunit of protein phosphatase type 1 (PP1) [118]. GADD34, together with PP1C, dephosphorylates eIF2α to resume translation of proteins [119, 120] (Fig. 1B). Resumed protein translation in stressed cells will eventually increase the ER protein load, further promoting ER stress-induced apoptosis [118, 121, 122]. The regulated IRE1-dependent decay (RIDD) pathway complements the general transcriptional and translational regulation during chronic ER stress (Fig. 1A) [28]. IRE1 mediates the degradation of newly targeted mRNAs or existing mRNAs at the ER translocon site to reduce overall ER protein load [123]. This activity results in the termination of protein synthesis at the ER and thus reduces protein overload within the ER [28, 29, 123]. In addition to its pro-survival properties, IRE1 additionally exhibits pro-apoptotic properties by phosphorylating c-Jun amino-terminal kinase (JNK) during chronic ER stress [124]. Activated JNK phosphorylates and suppresses the anti-apoptotic activity of the B-cell CLL/lymphoma 2 (BCL2) family of proteins and activate pro-apoptotic BCL2 homology domain 3 (BH3)-only proteins [125-127]. Despite its recognized pro-apoptotic role, JNK signalling can upregulate autophagy to promote cell survival [128]. Central to the pro-apoptotic role of PERK, CHOP induces a transcriptional programme that is similar to JNK-induced apoptosis signalling pathway. Additionally, CHOP activates the death receptor 5 (DR5) by sensitising cells to different conditions that trigger ER stress [118, 129]. To further amplify the transcriptional regulatory network of the UPR programme, ATF6(N) upregulates *CHOP* and *XBP1* [25, 112, 130]. Heterodimerisation of ATF6 with XBP1 has been shown to induce major ERAD components

400 under proteotoxic-induced ER stress, promoting the degradation of unfolded proteins by the ubiquitin
401 proteasome system [131]. In addition, CHOP expression was induced synergistically and additively by both
402 ATF6 and PERK pathways [109]. Together, these signalling cascades contribute to cell death when unfolded
403 proteins overload in the ER is not resolved.
404
405 *3.2. Transcriptional and non-transcriptional UPR programme upon LBS*
406 Although the three ER stress transducers are activated differently by LBS or proteotoxic stress, the subset of
407 the UPR transcriptional programme is conserved including genes that promote ER protein modification and
408 quality control, lipid metabolism, vesicle trafficking, and autophagy [8]. However, a large subset of the UPR^{LBS}
409 transcriptional programme diverge from proteotoxic stress. Prior to the identification of the LBS-specific
410 sensing mechanisms of IRE1, PERK, and ATF6, the UPR transcriptional programme that catered to LBS was
411 mostly deduced from lipotoxicity and metabolic syndromes [75, 132, 133].
412
413 To alleviate ER stress upon LBS, chemical chaperone tauroursodeoxycholic acid has been used in obese mice
414 models which correlate with a decrease in the size of adipocytes and serum levels of free fatty acids and
415 triglycerides [81, 134]. These studies indicate that repression of ER stress prevents metabolic abnormalities
416 related to obesity. *In vitro*, the saturated free fatty acid palmitic acid in combination with a stearoyl CoA
417 desaturase 1 (SCD1) inhibitor activate IRE1 and PERK through changes in the lipid bilayer properties [11]. In
418 addition, a recent study showed that ER stress is a major driver of lipotoxic cell death in palmitate-treated
419 human cells [135]. It correlates with the accumulation of saturated fatty acids, incorporated into glycerolipids,
420 which can be neutralised with a counterbalancing amount of unsaturated fatty acids [135]. UPR modulators
421 also play important regulatory roles in lipid homeostasis. For instance, XBP1 induces the synthesis of

422 phospholipids for the expansion of the ER membrane [136], and the overexpression of GADD34 in the liver
423 alleviates hepatosteatosis in mice [137]. Hence, the ER-spanning transmembrane domain of IRE1 and PERK
424 sense LBS and in turn results in the deployment of the UPR transcriptional programme.
425
426 Recently, efforts to distinguish the UPR^{LBS} transcriptional programme from UPR^{PT} have been made by several
427 groups. Accumulation of the sphingolipids DHS and DHC preferentially promotes ATF6-activated lipid
428 biosynthetic and metabolic genes with little effect on ER chaperone genes [83]. The differential UPR output
429 suggests that ATF6 distinguishes LBS and proteotoxic stress by either DHS/DHC-induced conformational
430 change or post-translational modification of ATF6 [83]. In contrast to ATF6, IRE1 or PERK transmembrane
431 domains replaced with an unrelated α-helix retain the capacity to be activated by both LBS and proteotoxic
432 stress. Therefore, the transcriptional UPR programme deployed might differ in response to different types of
433 LBS, in which ATF6 senses specific sphingolipids whereas IRE1 and PERK sense global changes within the
434 membrane [7, 84]. In *C. elegans*, we recently demonstrated that the transcriptome diverges dramatically
435 between UPR^{PT} and UPR^{LBS} [8]. In this study, UPR^{LBS} was induced by genetic attenuating *pmt-2*, an enzyme
436 required for the *de novo* biosynthesis of PC from PE, whereas UPR^{PT} was induced by tunicamycin. Overall,
437 2603 and 1745 genes were upregulated and downregulated, respectively, in *pmt-2*(RNAi) treated animals
438 compared to wildtype. On the other hand, tunicamycin-treated worms showed an upregulation of 1258 genes
439 and downregulation of 1473 genes. Consistent with previous findings that showed overlapping functions of
440 genes being regulated upon proteotoxic stress and LBS, there were 492 and 420 genes that were similarly
441 upregulated and downregulated, respectively, from both UPR^{PT} and UPR^{LBS} [8-10, 19, 104]. Further functional
442 analysis of 1069 UPR^{LBS}-regulated genes showed an enrichment of processes related to ER stress and
443 downregulation of genes related to translation initiation factors, a common characteristic observed upon UPR

444 activation. Additionally, individual branches of the UPR were found to regulate distinct processes including the
445 innate immunity response, lipid and fatty acid processes, as well as protein modifications and cell signalling
446 cascades [8]. Most of the genes that are upregulated by UPR^{LBS} were modulated by IRE-1 while PEK-1
447 regulated a small subset of genes, similar to the upregulated genes upon proteotoxic stress [104]. Moreover
448 446 genes were modulated by at least two of the three UPR branches, further suggesting compensatory roles
449 of one or more UPR transducers in the absence of the other. Possibly the plasticity of the UPR to modify its
450 output programme could be beneficial in curbing the development of diseases associated with lipid disruption
451 [138-141]. Together, these studies clearly point to a transcriptional programme curated to LBS. Further studies
452 should explore the specificity of UPR^{LBS}-induced genes to better understand their roles in LBS-related diseases.
453

454 **4. Conclusion remarks**

455 The UPR is a robust adaptive response to protect the ER and the cell against life-threatening intracellular
456 variations. In recent years, we learned that the UPR not only responds to the accumulation of unfolded proteins
457 at the ER but also to LBS. The three ER stress sensors are activated by both cues to deploy a broad-spectrum
458 compensatory pathway. Although the ER stress sensing mechanisms and the core of UPR-induced
459 upregulated genes are highly conserved, there are considerable variations across organisms and between cell
460 types. The significance of these differences and the influence on restoring ER homeostasis are largely
461 unexplored. As the UPR programme is essential during development and in certain cellular contexts, a better
462 understanding of these variations of the UPR programme will allow the development of targeted therapies
463 instead of shutting down an entire branch of the UPR.
464

465 To curb the progression of diseases associated with chronic ER stress, several compounds have been

identified to either inhibit UPR pro-survival effects or to attenuate ER stress levels. For instance, the compound GSK2656157 has been identified to inhibit the kinase activity of PERK which proved to be promising in targeting pancreatic adenocarcinoma and multiple myeloma in preclinical models [142]. However, it failed to progress further due to undesirable side effects that caused pancreatic β-cell loss and diabetes [143], similar to other inhibitors of PERK kinase activity. IRE1α, XBP1, and PERK inhibitors could be promising in treating diseases but their potential long-term side effects on the function of the pancreas, the immune system, and the liver need to be carefully assessed. In particular, the liver plays a critical role in cholesterol and lipid metabolism in addition of drug detoxification through the Ire1α/XBP1 axes [144-146]. Consequently, the UPR programme may be too broad to be inhibited from the upstream effectors. Thus, specific inhibition of a subset of downstream targets that are part of a specific UPR transcriptional programme may yield better success and potentially minimise undesirable long-term side effects. In order to identify such targets, substantial efforts should be made to characterise stress-specific UPR transcriptional programmes in the near future.

ACKNOWLEDGEMENTS

We are grateful to our lab members Nurulain Ho, Peter Jr Shyu, and Dr. Subhash Thalappilly for critical reading of the manuscript. This work was supported by the Nanyang Assistant Professorship programme from Nanyang Technological University and the Singapore Ministry of Education Academic Research Fund Tier 2 (MOE2018-T2-1-002).

FIGURE LEGENDS

Figure 1. Activation of the unfolded protein response (UPR). (A) In mammals, the accumulation of unfolded proteins or lipid bilayer stress (LBS) result in ER stress. (A) Upon sensing stress, IRE1α dimerisation or higher

oligomers from monomer, followed by trans-autophosphorylation. Subsequently, IRE1α splices *XBP1* mRNA, resulting in the translation of the transcription factor XBP1 which regulates a subset of UPR genes. Prolonged ER stress activates the regulated IRE1-dependent decay (RIDD), cleaving a subset of mRNA to decrease mRNA load. Both JNK and NF-kB are activated by IRE1α to initiate the transcription of proinflammatory genes. (B) PERK oligomerises and phosphorylates itself and eIF2α. eIF2α-P attenuates general protein translation while activating the translation of the transcription factor ATF4. Subsequently, ATF4 activates a subset of UPR genes including pro-apoptotic factor CHOP and GADD34. GADD34 promotes the dephosphorylation of eIF2α, resuming translation. (C) ATF6 is transported to the Golgi apparatus where the N-terminal transcription factor [ATF6(N)] is released by intramembrane proteolysis. ATF6(N) regulates a subset of UPR genes.

Figure 2. Models of ER stress transducers sensing mechanism. (A) The accumulation of unfolded protein in the ER promotes IRE1 oligomerisation, autophosphorylation, and consequently activation. In the absence of stress, molecular chaperone BiP binds IRE1 but will favourably assist in folding unfolded proteins. (B) Lipid bilayer stress (LBS) favours the bending of IRE1 amphipathic helix and transmembrane domain within the localised compressed lipid bilayer, promoting IRE1 activation. (C) In the absence of proteotoxic stress, ATF6 can be activated by LBS through the accumulation of dihydrosphingosine (DHS) or dihydroceramide (DHC). Potentially, DHS/DHC favour membrane curvature and thus the packing of ATF6 within COPII-mediated vesicle, resulting in the release of cleaved of cytosolic ATF6(N).

Figure 3. The deployed UPR programme is crafted to stress-specific-induced ER stress. The three ER stress sensors IRE1, PERK, and ATF6 activate their conserved linear pathways resulting in activation and inhibition of cellular responses. This response is fine-tuned through feedback regulation and thus become adapted to

510 the source of stress to either restore ER homeostasis, maintain viability, or induce cell death.

511

REFERENCES

[1] H.P. Harding, Y. Zhang, D. Ron, Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature, 397 (1999) 271-274.

[2] M.J. Gething, J. Sambrook, Protein folding in the cell, Nature, 355 (1992) 33-45.

[3] K. Mori, Tripartite management of unfolded proteins in the endoplasmic reticulum, Cell, 101 (2000) 451-454.

[4] J. Hollien, Evolution of the unfolded protein response, Biochim Biophys Acta, 1833 (2013) 2458-2463.

[5] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat Rev Mol Cell Biol, 8 (2007) 519-529.

[6] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, Science, 334 (2011) 1081-1086.

[7] K. Halbleib, K. Pesek, R. Covino, H.F. Hofbauer, D. Wunnicke, I. Hanelt, G. Hummer, R. Ernst, Activation of the Unfolded Protein Response by Lipid Bilayer Stress, Mol Cell, 67 (2017) 673-684 e678.

[8] J.H. Koh, L. Wang, C. Beaudoin-Chabot, G. Thibault, Lipid bilayer stress-activated IRE-1 modulates autophagy during endoplasmic reticulum stress, J Cell Sci, 131 (2018).

[9] T. Promlek, Y. Ishiwata-Kimata, M. Shido, M. Sakuramoto, K. Kohno, Y. Kimata, Membrane aberrancy and unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways, Mol Biol Cell, 22 (2011) 3520-3532.

[10] G. Thibault, G. Shui, W. Kim, G.C. McAlister, N. Ismail, S.P. Gygi, M.R. Wenk, D.T. Ng, The membrane stress response buffers lethal effects of lipid disequilibrium by reprogramming the protein homeostasis network, Mol Cell, 48 (2012) 16-27.

[11] R. Volmer, K. van der Ploeg, D. Ron, Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains, Proc Natl Acad Sci U S A, 110 (2013) 4628-4633.

[12] M.A. Surma, C. Klose, D. Peng, M. Shales, C. Mrejen, A. Stefanko, H. Braberg, D.E. Gordon, D. Vorkel, C.S. Ejsing, R. Farese, Jr., K. Simons, N.J. Krogan, R. Ernst, A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress, Mol Cell, 51 (2013) 519-530.

[13] L. Pineau, J. Colas, S. Dupont, L. Beney, P. Fleurat-Lessard, J.M. Berjeaud, T. Berges, T. Ferreira, Lipid-induced ER stress: synergistic effects of sterols and saturated fatty acids, Traffic, 10 (2009) 673-690.

[14] T. Kawahara, H. Yanagi, T. Yura, K. Mori, Endoplasmic reticulum stress-induced mRNA splicing permits synthesis of transcription factor Hac1p/Ern4p that activates the unfolded protein response, Mol Biol Cell, 8 (1997) 1845-1862.

[15] J.S. Cox, C.E. Shamu, P. Walter, Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase, Cell, 73 (1993) 1197-1206.

[16] C.E. Shamu, P. Walter, Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the endoplasmic reticulum to the nucleus, EMBO J, 15 (1996) 3028-3039.

[17] Y. Kimata, Y. Ishiwata-Kimata, T. Ito, A. Hirata, T. Suzuki, D. Oikawa, M. Takeuchi, K. Kohno, Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins, J Cell Biol, 179 (2007) 75-86.

[18] H. Li, A.V. Korennykh, S.L. Behrman, P. Walter, Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering, Proc Natl Acad Sci U S A, 107 (2010) 16113-16118.

553 [19] K.J. Travers, C.K. Patil, L. Wodicka, D.J. Lockhart, J.S. Weissman, P. Walter, Functional and genomic
554 analyses reveal an essential coordination between the unfolded protein response and ER-associated
555 degradation, Cell, 101 (2000) 249-258.
556 [20] G. Thibault, N. Ismail, D.T. Ng, The unfolded protein response supports cellular robustness as a broad-
557 spectrum compensatory pathway, Proc Natl Acad Sci U S A, 108 (2011) 20597-20602.
558 [21] X.Z. Wang, H.P. Harding, Y. Zhang, E.M. Jolicoeur, M. Kuroda, D. Ron, Cloning of mammalian Ire1 reveals
559 diversity in the ER stress responses, EMBO J, 17 (1998) 5708-5717.
560 [22] W. Tirasophon, A.A. Welihinda, R.J. Kaufman, A stress response pathway from the endoplasmic reticulum
561 to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells,
562 Genes Dev, 12 (1998) 1812-1824.
563 [23] A. Bertolotti, X. Wang, I. Novoa, R. Jungreis, K. Schlessinger, J.H. Cho, A.B. West, D. Ron, Increased
564 sensitivity to dextran sodium sulfate colitis in IRE1beta-deficient mice, J Clin Invest, 107 (2001) 585-593.
565 [24] M. Calfon, H. Zeng, F. Urano, J.H. Till, S.R. Hubbard, H.P. Harding, S.G. Clark, D. Ron, IRE1 couples
566 endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA, Nature, 415 (2002) 92-96.
567 [25] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada, K. Mori, XBP1 mRNA is induced by ATF6 and spliced by
568 IRE1 in response to ER stress to produce a highly active transcription factor, Cell, 107 (2001) 881-891.
569 [26] S. Kanda, K. Yanagitani, Y. Yokota, Y. Esaki, K. Kohno, Autonomous translational pausing is required for
570 XBP1u mRNA recruitment to the ER via the SRP pathway, Proc Natl Acad Sci U S A, 113 (2016) E5886-
571 E5895.
572 [27] R. Plumb, Z.R. Zhang, S. Appathurai, M. Mariappan, A functional link between the co-translational protein
573 translocation pathway and the UPR, Elife, 4 (2015).
574 [28] J. Hollien, J.S. Weissman, Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein
575 response, Science, 313 (2006) 104-107.
576 [29] J. Hollien, J.H. Lin, H. Li, N. Stevens, P. Walter, J.S. Weissman, Regulated Ire1-dependent decay of
577 messenger RNAs in mammalian cells, J Cell Biol, 186 (2009) 323-331.
578 [30] Y. Ma, J.W. Brewer, J.A. Diehl, L.M. Hendershot, Two distinct stress signaling pathways converge upon
579 the CHOP promoter during the mammalian unfolded protein response, J Mol Biol, 318 (2002) 1351-1365.
580 [31] A.J. Schindler, R. Schekman, In vitro reconstitution of ER-stress induced ATF6 transport in COPII vesicles,
581 Proc Natl Acad Sci U S A, 106 (2009) 17775-17780.
582 [32] S. Nadanaka, T. Okada, H. Yoshida, K. Mori, Role of disulfide bridges formed in the luminal domain of
583 ATF6 in sensing endoplasmic reticulum stress, Mol Cell Biol, 27 (2007) 1027-1043.
584 [33] M.A. Surani, Glycoprotein synthesis and inhibition of glycosylation by tunicamycin in preimplantation
585 mouse embryos: compaction and trophoblast adhesion, Cell, 18 (1979) 217-227.
586 [34] J.K. Hakulinen, J. Hering, G. Branden, H. Chen, A. Snijder, M. Ek, P. Johansson, MraY-antibiotic complex
587 reveals details of tunicamycin mode of action, Nat Chem Biol, 13 (2017) 265-267.
588 [35] J. Yoo, E.H. Mashalidis, A.C.Y. Kuk, K. Yamamoto, B. Kaeser, S. Ichikawa, S.Y. Lee, GlcNAc-1-P-
589 transferase-tunicamycin complex structure reveals basis for inhibition of N-glycosylation, Nat Struct Mol Biol,
590 25 (2018) 217-224.
591 [36] Y. Kozutsumi, M. Segal, K. Normington, M.J. Gething, J. Sambrook, The presence of malfolded proteins
592 in the endoplasmic reticulum signals the induction of glucose-regulated proteins, Nature, 332 (1988) 462-464.
593 [37] A.J. Dorner, L.C. Wasley, P. Raney, S. Haugejorden, M. Green, R.J. Kaufman, The stress response in
594 Chinese hamster ovary cells. Regulation of ERp72 and protein disulfide isomerase expression and secretion,
595 J Biol Chem, 265 (1990) 22029-22034.

[38] K. Mori, W. Ma, M.J. Gething, J. Sambrook, A transmembrane protein with a cdc2+/CDC28-related kinase activity is required for signaling from the ER to the nucleus, Cell, 74 (1993) 743-756.
[39] J.S. Cox, P. Walter, A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response, Cell, 87 (1996) 391-404.
[40] C.R. Prostko, M.A. Brostrom, C.O. Brostrom, Reversible phosphorylation of eukaryotic initiation factor 2 alpha in response to endoplasmic reticular signaling, Mol Cell Biochem, 127-128 (1993) 255-265.
[41] W.L. Wong, M.A. Brostrom, G. Kuznetsov, D. Gmitter-Yellen, C.O. Brostrom, Inhibition of protein synthesis and early protein processing by thapsigargin in cultured cells, Biochem J, 289 (Pt 1) (1993) 71-79.
[42] C.O. Brostrom, M.A. Brostrom, Regulation of translational initiation during cellular responses to stress, Prog Nucleic Acid Res Mol Biol, 58 (1998) 79-125.
[43] K. Haze, H. Yoshida, H. Yanagi, T. Yura, K. Mori, Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress, Mol Biol Cell, 10 (1999) 3787-3799.
[44] K. Haze, T. Okada, H. Yoshida, H. Yanagi, T. Yura, M. Negishi, K. Mori, Identification of the G13 (cAMP-response-element-binding protein-related protein) gene product related to activating transcription factor 6 as a transcriptional activator of the mammalian unfolded protein response, Biochem J, 355 (2001) 19-28.
[45] A. Bertolotti, Y. Zhang, L.M. Hendershot, H.P. Harding, D. Ron, Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response, Nat Cell Biol, 2 (2000) 326-332.
[46] K. Okamura, Y. Kimata, H. Higashio, A. Tsuru, K. Kohno, Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast, Biochem Biophys Res Commun, 279 (2000) 445-450.
[47] Y. Kimata, D. Oikawa, Y. Shimizu, Y. Ishiwata-Kimata, K. Kohno, A role for BiP as an adjustor for the endoplasmic reticulum stress-sensing protein Ire1, J Cell Biol, 167 (2004) 445-456.
[48] M. Carrara, F. Prischi, P.R. Nowak, M.C. Kopp, M.M. Ali, Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling, Elife, 4 (2015).
[49] M.C. Kopp, P.R. Nowak, N. Larburu, C.J. Adams, M.M. Ali, In vitro FRET analysis of IRE1 and BiP association and dissociation upon endoplasmic reticulum stress, Elife, 7 (2018).
[50] J.J. Credle, J.S. Finer-Moore, F.R. Papa, R.M. Stroud, P. Walter, On the mechanism of sensing unfolded protein in the endoplasmic reticulum, Proc Natl Acad Sci U S A, 102 (2005) 18773-18784.
[51] D. Oikawa, Y. Kimata, K. Kohno, Self-association and BiP dissociation are not sufficient for activation of the ER stress sensor Ire1, J Cell Sci, 120 (2007) 1681-1688.
[52] B.M. Gardner, P. Walter, Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response, Science, 333 (2011) 1891-1894.
[53] G.E. Karagoz, D. Acosta-Alvear, H.T. Nguyen, C.P. Lee, F. Chu, P. Walter, An unfolded protein-induced conformational switch activates mammalian IRE1, Elife, 6 (2017).
[54] K. Ma, K.M. Vattem, R.C. Wek, Dimerization and release of molecular chaperone inhibition facilitate activation of eukaryotic initiation factor-2 kinase in response to endoplasmic reticulum stress, J Biol Chem, 277 (2002) 18728-18735.
[55] A.A. Welihinda, R.J. Kaufman, The unfolded protein response pathway in Saccharomyces cerevisiae. Oligomerization and trans-phosphorylation of Ire1p (Ern1p) are required for kinase activation, J Biol Chem, 271 (1996) 18181-18187.
[56] C.Y. Liu, M. Schroder, R.J. Kaufman, Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum, J Biol Chem, 275 (2000) 24881-24885.

[57] M.M. Ali, T. Bagratuni, E.L. Davenport, P.R. Nowak, M.C. Silva-Santisteban, A. Hardcastle, C. McAndrews, M.G. Rowlands, G.J. Morgan, W. Aherne, I. Collins, F.E. Davies, L.H. Pearl, Structure of the Ire1 autophosphorylation complex and implications for the unfolded protein response, EMBO J, 30 (2011) 894-905.
[58] F. Prischi, P.R. Nowak, M. Carrara, M.M. Ali, Phosphoregulation of Ire1 RNase splicing activity, Nat Commun, 5 (2014) 3554.
[59] M. Carrara, F. Prischi, P.R. Nowak, M.M. Ali, Crystal structures reveal transient PERK luminal domain tetramerization in endoplasmic reticulum stress signaling, EMBO J, 34 (2015) 1589-1600.
[60] P. Wang, J. Li, J. Tao, B. Sha, The luminal domain of the ER stress sensor protein PERK binds misfolded proteins and thereby triggers PERK oligomerization, J Biol Chem, 293 (2018) 4110-4121.
[61] B. Sha, S. Lee, D.M. Cyr, The crystal structure of the peptide-binding fragment from the yeast Hsp40 protein Sis1, Structure, 8 (2000) 799-807.
[62] J. Li, X. Qian, B. Sha, The crystal structure of the yeast Hsp40 Ydj1 complexed with its peptide substrate, Structure, 11 (2003) 1475-1483.
[63] A.L. Horwich, W.A. Fenton, E. Chapman, G.W. Farr, Two families of chaperonin: physiology and mechanism, Annu Rev Cell Dev Biol, 23 (2007) 115-145.
[64] N. Jaya, V. Garcia, E. Vierling, Substrate binding site flexibility of the small heat shock protein molecular chaperones, Proc Natl Acad Sci U S A, 106 (2009) 15604-15609.
[65] M. Taipale, D.F. Jarosz, S. Lindquist, HSP90 at the hub of protein homeostasis: emerging mechanistic insights, Nat Rev Mol Cell Biol, 11 (2010) 515-528.
[66] D.R. Southworth, D.A. Agard, Client-loading conformation of the Hsp90 molecular chaperone revealed in the cryo-EM structure of the human Hsp90:Hop complex, Mol Cell, 42 (2011) 771-781.
[67] H. Yoshida, K. Haze, H. Yanagi, T. Yura, K. Mori, Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors, J Biol Chem, 273 (1998) 33741-33749.
[68] X. Chen, J. Shen, R. Prywes, The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi, J Biol Chem, 277 (2002) 13045-13052.
[69] J. Shen, X. Chen, L. Hendershot, R. Prywes, ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals, Dev Cell, 3 (2002) 99-111.
[70] J. Shen, E.L. Snapp, J. Lippincott-Schwartz, R. Prywes, Stable binding of ATF6 to BiP in the endoplasmic reticulum stress response, Mol Cell Biol, 25 (2005) 921-932.
[71] S. Nadanaka, H. Yoshida, K. Mori, Reduction of disulfide bridges in the lumenal domain of ATF6 in response to glucose starvation, Cell Struct Funct, 31 (2006) 127-134.
[72] Y. Sato, S. Nadanaka, T. Okada, K. Okawa, K. Mori, Luminal domain of ATF6 alone is sufficient for sensing endoplasmic reticulum stress and subsequent transport to the Golgi apparatus, Cell Struct Funct, 36 (2011) 35-47.
[73] S.A. Jesch, X. Zhao, M.T. Wells, S.A. Henry, 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 (2005) 9106-9118.
[74] S.A. Jesch, P. Liu, X. Zhao, M.T. Wells, S.A. Henry, Multiple endoplasmic reticulum-to-nucleus signaling pathways coordinate phospholipid metabolism with gene expression by distinct mechanisms, J Biol Chem, 281 (2006) 24070-24083.
[75] S. Fu, L. Yang, P. Li, O. Hofmann, L. Dicker, W. Hide, X. Lin, S.M. Watkins, A.R. Ivanov, G.S. Hotamisligil, Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity, Nature, 473 (2011) 528-531.

[76] W. Cui, J. Ma, X. Wang, W. Yang, J. Zhang, Q. Ji, Free fatty acid induces endoplasmic reticulum stress and apoptosis of beta-cells by Ca2+/calpain-2 pathways, PLoS One, 8 (2013) e59921.
[77] Q. Xie, V.I. Khaoustov, C.C. Chung, J. Sohn, B. Krishnan, D.E. Lewis, B. Yoffe, Effect of tauroursodeoxycholic acid on endoplasmic reticulum stress-induced caspase-12 activation, Hepatology, 36 (2002) 592-601.
[78] C.R. Brown, L.Q. Hong-Brown, J. Biwersi, A.S. Verkman, W.J. Welch, Chemical chaperones correct the mutant phenotype of the delta F508 cystic fibrosis transmembrane conductance regulator protein, Cell Stress Chaperones, 1 (1996) 117-125.
[79] W.J. Welch, C.R. Brown, Influence of molecular and chemical chaperones on protein folding, Cell Stress Chaperones, 1 (1996) 109-115.
[80] T. Kolter, M. Wendeler, Chemical chaperones--a new concept in drug research, Chembiochem, 4 (2003) 260-264.
[81] U. Ozcan, E. Yilmaz, L. Ozcan, M. Furuhashi, E. Vaillancourt, R.O. Smith, C.Z. Gorgun, G.S. Hotamisligil, Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes, Science, 313 (2006) 1137-1140.
[82] T.W. Loo, D.M. Clarke, Chemical and pharmacological chaperones as new therapeutic agents, Expert Rev Mol Med, 9 (2007) 1-18.
[83] A.B. Tam, L.S. Roberts, V. Chandra, I.G. Rivera, D.K. Nomura, D.J. Forbes, M. Niwa, The UPR Activator ATF6 Responds to Proteotoxic and Lipotoxic Stress by Distinct Mechanisms, Dev Cell, 46 (2018) 327-343 e327.
[84] N. Kono, N. Amin-Wetzel, D. Ron, Generic membrane-spanning features endow IRE1alpha with responsiveness to membrane aberrancy, Mol Biol Cell, 28 (2017) 2318-2332.
[85] F.X. Contreras, A.M. Ernst, P. Haberkant, P. Bjorkholm, E. Lindahl, B. Gonen, C. Tischer, A. Elofsson, G. von Heijne, C. Thiele, R. Pepperkok, F. Wieland, B. Brugger, Molecular recognition of a single sphingolipid species by a protein's transmembrane domain, Nature, 481 (2012) 525-529.
[86] J.E. Stukey, V.M. McDonough, C.E. Martin, Isolation and characterization of OLE1, a gene affecting fatty acid desaturation from Saccharomyces cerevisiae, J Biol Chem, 264 (1989) 16537-16544.
[87] S. Zhang, Y. Skalsky, D.J. Garfinkel, MGA2 or SPT23 is required for transcription of the delta9 fatty acid desaturase gene, OLE1, and nuclear membrane integrity in Saccharomyces cerevisiae, Genetics, 151 (1999) 473-483.
[88] T. Hoppe, K. Matuschewski, M. Rape, S. Schlenker, H.D. Ulrich, S. Jentsch, Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing, Cell, 102 (2000) 577-586.
[89] M. Rape, T. Hoppe, I. Gorr, M. Kalocay, H. Richly, S. Jentsch, Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone, Cell, 107 (2001) 667-677.
[90] N. Shcherbik, D.S. Haines, Cdc48p(Npl4p/Ufd1p) binds and segregates membrane-anchored/tethered complexes via a polyubiquitin signal present on the anchors, Mol Cell, 25 (2007) 385-397.
[91] R. Covino, S. Ballweg, C. Stordeur, J.B. Michaelis, K. Puth, F. Wernig, A. Bahrami, A.M. Ernst, G. Hummer, R. Ernst, A Eukaryotic Sensor for Membrane Lipid Saturation, Mol Cell, 63 (2016) 49-59.
[92] J.L. Goldstein, R.A. DeBose-Boyd, M.S. Brown, Protein sensors for membrane sterols, Cell, 124 (2006) 35-46.
[93] P.J. Espenshade, A.L. Hughes, Regulation of sterol synthesis in eukaryotes, Annu Rev Genet, 41 (2007) 401-427.

[94] J. Ye, R.B. Rawson, R. Komuro, X. Chen, U.P. Dave, R. Prywes, M.S. Brown, J.L. Goldstein, ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs, Mol Cell, 6 (2000) 1355-1364.
[95] M.S. Brown, J.L. Goldstein, The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor, Cell, 89 (1997) 331-340.
[96] C.L. Theesfeld, D. Pourmand, T. Davis, R.M. Garza, R.Y. Hampton, The sterol-sensing domain (SSD) directly mediates signal-regulated endoplasmic reticulum-associated degradation (ERAD) of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase isozyme Hmg2, J Biol Chem, 286 (2011) 26298-26307.
[97] K.A. Moore, J. Hollien, The unfolded protein response in secretory cell function, Annu Rev Genet, 46 (2012) 165-183.
[98] A.H. Lee, G.C. Chu, N.N. Iwakoshi, L.H. Glimcher, XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands, EMBO J, 24 (2005) 4368-4380.
[99] D. Acosta-Alvear, Y. Zhou, A. Blais, M. Tsikitis, N.H. Lents, C. Arias, C.J. Lennon, Y. Kluger, B.D. Dynlacht, XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks, Mol Cell, 27 (2007) 53-66.
[100] H. Ilieva, M. Polymenidou, D.W. Cleveland, Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond, J Cell Biol, 187 (2009) 761-772.
[101] J.J. Rodvold, K.T. Chiu, N. Hiramatsu, J.K. Nussbacher, V. Galimberti, N.R. Mahadevan, K. Willert, J.H. Lin, M. Zanetti, Intercellular transmission of the unfolded protein response promotes survival and drug resistance in cancer cells, Sci Signal, 10 (2017).
[102] R.C. Taylor, A. Dillin, XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity, Cell, 153 (2013) 1435-1447.
[103] J.R. Daniele, R. Higuchi-Sanabria, V. Ramachandran, M. Sanchez, J. Durieux, S.U. Tronnes, J.W. Paul, D.J. Esping, S. Monshietehadi, M.G. Metcalf, A. Dillin, A non-canonical arm of UPR^{ER} mediates longevity through ER remodeling and lipophagy, bioRxiv, (2018) 471177.
[104] X. Shen, R.E. Ellis, K. Sakaki, R.J. Kaufman, Genetic interactions due to constitutive and inducible gene regulation mediated by the unfolded protein response in C. elegans, PLoS Genet, 1 (2005) e37.
[105] M.C. Jonikas, S.R. Collins, V. Denic, E. Oh, E.M. Quan, V. Schmid, J. Weibezahn, B. Schwappach, P. Walter, J.S. Weissman, M. Schuldiner, Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum, Science, 323 (2009) 1693-1697.
[106] P. Lajoie, R.D. Moir, I.M. Willis, E.L. Snapp, Kar2p availability defines distinct forms of endoplasmic reticulum stress in living cells, Mol Biol Cell, 23 (2012) 955-964.
[107] N.S. Hou, A. Gutschmidt, D.Y. Choi, K. Pather, X. Shi, J.L. Watts, T. Hoppe, S. Taubert, Activation of the endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis in vivo, Proc Natl Acad Sci U S A, 111 (2014) E2271-2280.
[108] C. Hetz, E. Chevet, H.P. Harding, Targeting the unfolded protein response in disease, Nat Rev Drug Discov, 12 (2013) 703-719.
[109] T. Okada, H. Yoshida, R. Akazawa, M. Negishi, K. Mori, 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 (2002) 585-594.
[110] A.H. Lee, N.N. Iwakoshi, L.H. Glimcher, XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response, Mol Cell Biol, 23 (2003) 7448-7459.

[111] K. Yamamoto, H. Yoshida, K. Kokame, R.J. Kaufman, K. Mori, Differential contributions of ATF6 and XBP1 to the activation of endoplasmic reticulum stress-responsive cis-acting elements ERSE, UPRE and ERSE-II, J Biochem, 136 (2004) 343-350.
[112] Y. Adachi, K. Yamamoto, T. Okada, H. Yoshida, A. Harada, K. Mori, ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum, Cell Struct Funct, 33 (2008) 75-89.
[113] J.S. Shen, X. Chen, L. Hendershot, R. Prywes, ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of golgi localization signals, Developmental Cell, 3 (2002) 99-111.
[114] S. Takayanagi, R. Fukuda, Y. Takeuchi, S. Tsukada, K. Yoshida, Gene regulatory network of unfolded protein response genes in endoplasmic reticulum stress, Cell Stress Chaperones, 18 (2013) 11-23.
[115] S.B. Cullinan, J.A. Diehl, PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress, J Biol Chem, 279 (2004) 20108-20117.
[116] M.S. Hayden, S. Ghosh, Signaling to NF-kappaB, Genes Dev, 18 (2004) 2195-2224.
[117] A.B. Tam, E.L. Mercado, A. Hoffmann, M. Niwa, ER stress activates NF-kappaB by integrating functions of basal IKK activity, IRE1 and PERK, PLoS One, 7 (2012) e45078.
[118] S.J. Marciniak, C.Y. Yun, S. Oyadomari, I. Novoa, Y. Zhang, R. Jungreis, K. Nagata, H.P. Harding, D. Ron, CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum, Genes Dev, 18 (2004) 3066-3077.
[119] I. Novoa, H. Zeng, H.P. Harding, D. Ron, Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha, J Cell Biol, 153 (2001) 1011-1022.
[120] Y. Ma, L.M. Hendershot, Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress, J Biol Chem, 278 (2003) 34864-34873.
[121] M.H. Brush, D.C. Weiser, S. Shenolikar, Growth arrest and DNA damage-inducible protein GADD34 targets protein phosphatase 1 alpha to the endoplasmic reticulum and promotes dephosphorylation of the alpha subunit of eukaryotic translation initiation factor 2, Mol Cell Biol, 23 (2003) 1292-1303.
[122] D.E. Feldman, V. Chauhan, A.C. Koong, The unfolded protein response: a novel component of the hypoxic stress response in tumors, Mol Cancer Res, 3 (2005) 597-605.
[123] D. Acosta-Alvear, G.E. Karagoz, F. Frohlich, H. Li, T.C. Walther, P. Walter, The unfolded protein response and endoplasmic reticulum protein targeting machineries converge on the stress sensor IRE1, Elife, 7 (2018).
[124] F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H.P. Harding, D. Ron, Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1, Science, 287 (2000) 664-666.
[125] R.J. Davis, Signal transduction by the JNK group of MAP kinases, Cell, 103 (2000) 239-252.
[126] K. Lei, R.J. Davis, JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis, Proc Natl Acad Sci U S A, 100 (2003) 2432-2437.
[127] M.C. Bassik, L. Scorrano, S.A. Oakes, T. Pozzan, S.J. Korsmeyer, Phosphorylation of BCL-2 regulates ER Ca2+ homeostasis and apoptosis, EMBO J, 23 (2004) 1207-1216.
[128] Y. Wei, S. Pattingre, S. Sinha, M. Bassik, B. Levine, JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy, Mol Cell, 30 (2008) 678-688.
[129] H. Yamaguchi, H.G. Wang, CHOP is involved in endoplasmic reticulum stress-induced apoptosis by enhancing DR5 expression in human carcinoma cells, J Biol Chem, 279 (2004) 45495-45502.
[130] H. Yoshida, T. Okada, K. Haze, H. Yanagi, T. Yura, M. Negishi, K. Mori, 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 (2000) 6755-6767.

[131] K. Yamamoto, T. Sato, T. Matsui, M. Sato, T. Okada, H. Yoshida, A. Harada, K. Mori, Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1, Dev Cell, 13 (2007) 365-376.
[132] B. Feng, P.M. Yao, Y. Li, C.M. Devlin, D. Zhang, H.P. Harding, M. Sweeney, J.X. Rong, G. Kuriakose, E.A. Fisher, A.R. Marks, D. Ron, I. Tabas, The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages, Nat Cell Biol, 5 (2003) 781-792.
[133] D.A. Cunha, P. Hekerman, L. Ladriere, A. Bazarra-Castro, F. Ortis, M.C. Wakeham, F. Moore, J. Rasschaert, A.K. Cardozo, E. Bellomo, L. Overbergh, C. Mathieu, R. Lupi, T. Hai, A. Herchuelz, P. Marchetti, G.A. Rutter, D.L. Eizirik, M. Cnop, Initiation and execution of lipotoxic ER stress in pancreatic beta-cells, J Cell Sci, 121 (2008) 2308-2318.
[134] Y. Chen, Z. Wu, S. Zhao, R. Xiang, Chemical chaperones reduce ER stress and adipose tissue inflammation in high fat diet-induced mouse model of obesity, Sci Rep, 6 (2016) 27486.
[135] M. Piccolis, L.M. Bond, M. Kampmann, P. Pulimeno, C. Chitraju, C.B.K. Jayson, L.P. Vaites, S. Boland, Z.W. Lai, K.R. Gabriel, S.D. Elliott, J.A. Paulo, J.W. Harper, J.S. Weissman, T.C. Walther, R.V. Farese, Jr., Probing the Global Cellular Responses to Lipotoxicity Caused by Saturated Fatty Acids, Mol Cell, 74 (2019) 32-44 e38.
[136] R. Sriburi, S. Jackowski, K. Mori, J.W. Brewer, XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum, J Cell Biol, 167 (2004) 35-41.
[137] S. Oyadomari, H.P. Harding, Y. Zhang, M. Oyadomari, D. Ron, Dephosphorylation of translation initiation factor 2alpha enhances glucose tolerance and attenuates hepatosteatosis in mice, Cell metabolism, 7 (2008) 520-532.
[138] P. Puri, R.A. Baillie, M.M. Wiest, F. Mirshahi, J. Choudhury, O. Cheung, C. Sargeant, M.J. Contos, A.J. Sanyal, A lipidomic analysis of nonalcoholic fatty liver disease, Hepatology, 46 (2007) 1081-1090.
[139] D.G. Tiniakos, M.B. Vos, E.M. Brunt, Nonalcoholic fatty liver disease: pathology and pathogenesis, Annu Rev Pathol, 5 (2010) 145-171.
[140] B.M. Arendt, D.W. Ma, B. Simons, S.A. Noureldin, G. Therapondos, M. Guindi, M. Sherman, J.P. Allard, Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine to phosphatidylethanolamine, Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme, 38 (2013) 334-340.
[141] I. Doycheva, K.D. Watt, G. Rifai, R. Abou Mrad, R. Lopez, N.N. Zein, W.D. Carey, N. Alkhouri, Increasing Burden of Chronic Liver Disease Among Adolescents and Young Adults in the USA: A Silent Epidemic, Dig Dis Sci, 62 (2017) 1373-1380.
[142] C. Atkins, Q. Liu, E. Minthorn, S.Y. Zhang, D.J. Figueroa, K. Moss, T.B. Stanley, B. Sanders, A. Goetz, N. Gaul, A.E. Choudhry, H. Alsaid, B.M. Jucker, J.M. Axten, R. Kumar, Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity, Cancer Res, 73 (2013) 1993-2002.
[143] S.A. Oakes, Endoplasmic reticulum proteostasis: a key checkpoint in cancer, Am J Physiol Cell Physiol, 312 (2017) C93-C102.
[144] J.S. So, K.Y. Hur, M. Tarrio, V. Ruda, M. Frank-Kamenetsky, K. Fitzgerald, V. Koteliansky, A.H. Lichtman, T. Iwawaki, L.H. Glimcher, A.H. Lee, Silencing of lipid metabolism genes through IRE1alpha-mediated mRNA decay lowers plasma lipids in mice, Cell metabolism, 16 (2012) 487-499.
[145] S. Fu, S.M. Watkins, G.S. Hotamisligil, The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling, Cell metabolism, 15 (2012) 623-634.

854 [146] K.Y. Hur, J.S. So, V. Ruda, M. Frank-Kamenetsky, K. Fitzgerald, V. Koteliansky, T. Iwawaki, L.H.
855 Glimcher, A.H. Lee, IRE1alpha activation protects mice against acetaminophen-induced hepatotoxicity, J Exp
856 Med, 209 (2012) 307-318.

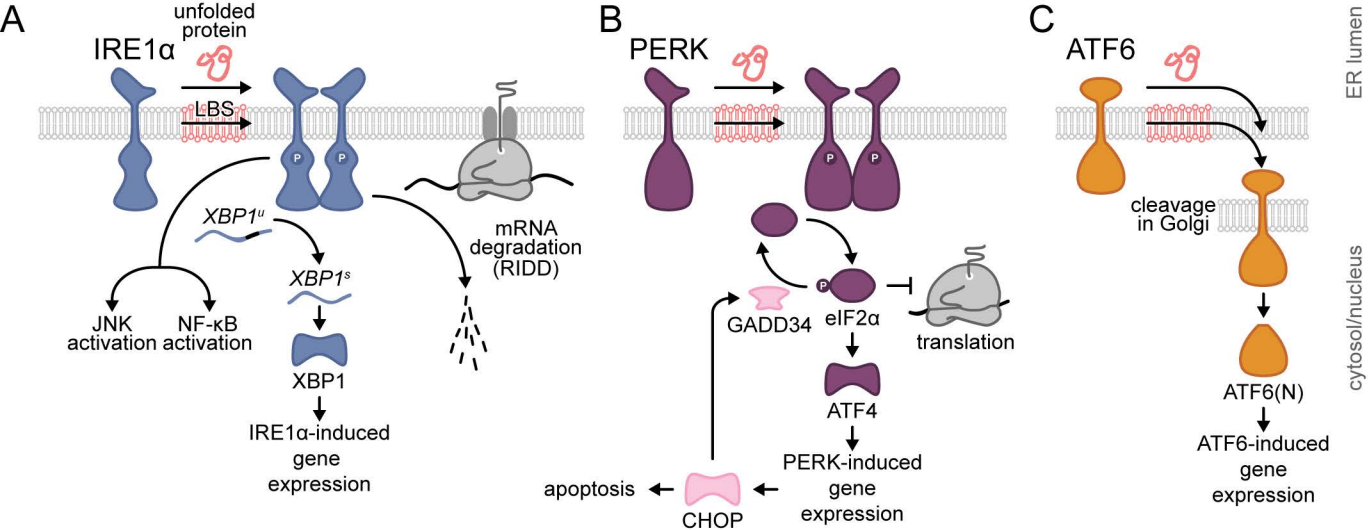
A
unfolded protein
IRE1α
LBS
P
P
XBP1u
XBP1s
JNK activation
NF-κB activation
XBP1
IRE1α-induced gene expression
mRNA degradation (RIDD)
B
PERK
P
P
P
GADD34
eIF2α
translation
ATF4
PERK-induced gene expression
apoptosis
CHOP
C
ATF6
cleavage in Golgi
ATF6(N)
ATF6-induced gene expression
ER lumen
cytosol/nucleus

Figure 1

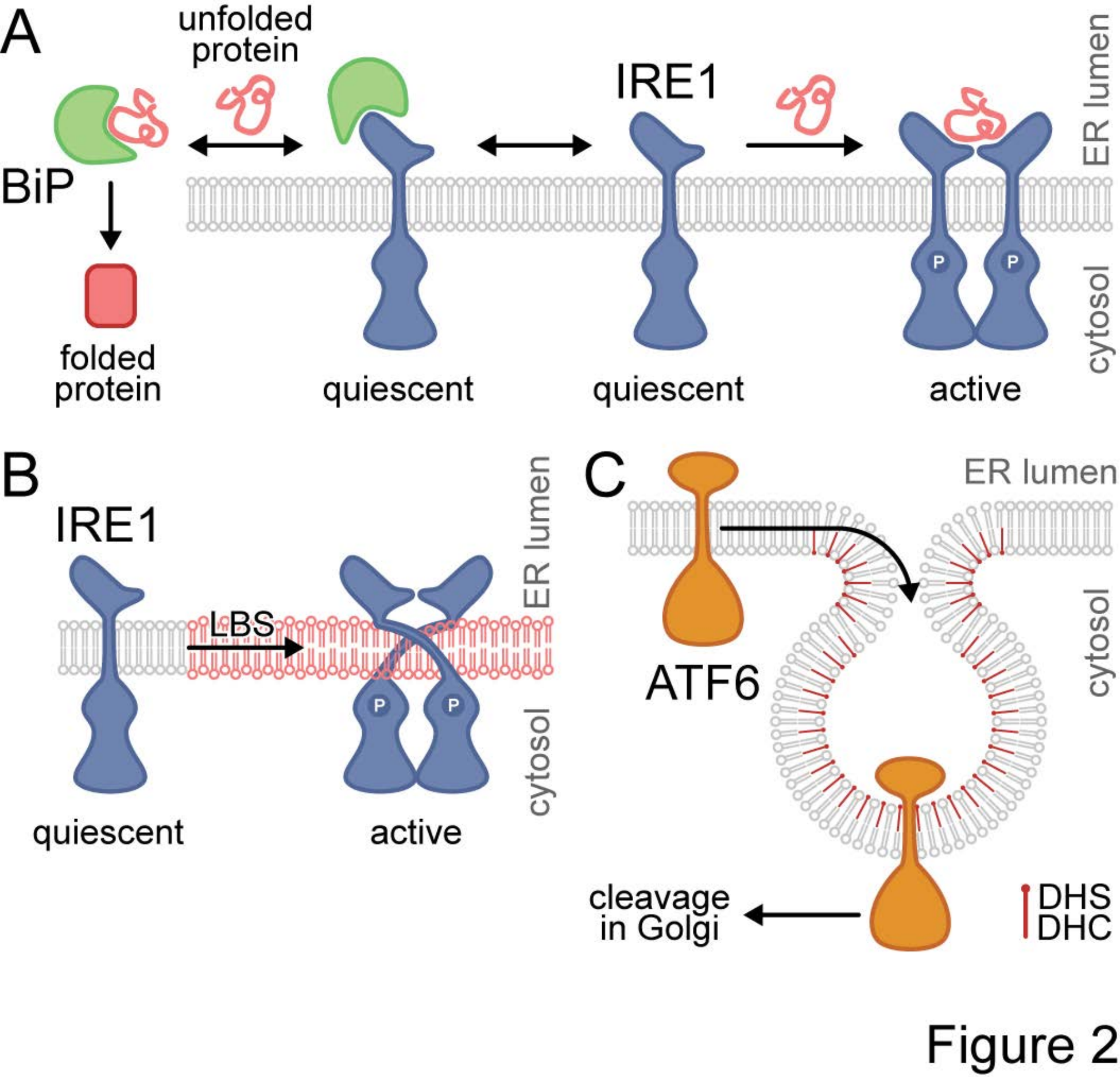
A
unfolded protein
IRE1
BiP
ER lumen
cytosol
P
P
folded protein
quiescent
quiescent
active
B
IRE1
LBS
ER lumen
cytosol
P
P
quiescent
active
C
ER lumen
cytosol
ATF6
cleavage in Golgi
DHS
DHC

Figure 2

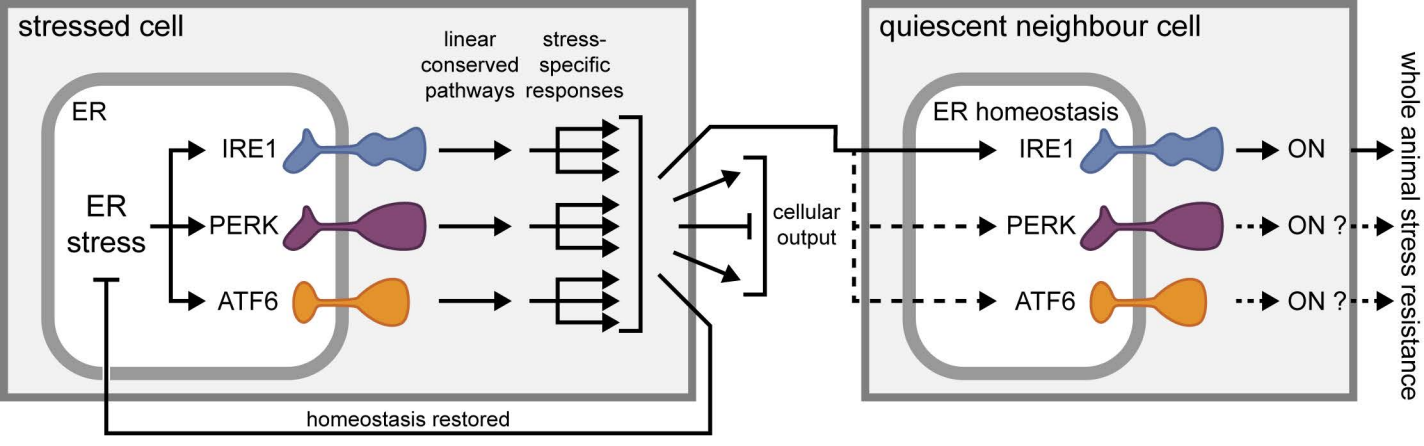
stressed cell
linear conserved pathways
stress-specific responses
ER
IRE1
ER stress
PERK
ATF6
cellular output
quiescent neighbour cell
ER homeostasis
IRE1
ON
PERK
ON ?
ATF6
ON ?
whole animal stress resistance
homeostasis restored

Figure 3