

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

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

43 **ABSTRACT**

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

54

55 **KEYWORDS**

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

58

59 **HIGHLIGHTS**

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

63

64 **ABBREVIATIONS**

65 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.

70 **1. INTRODUCTION**

71 The unfolded protein response (UPR) is a eukaryotic stress response pathway canonically activated by
72 endoplasmic reticulum (ER) stress. Equipped with different mechanisms including translational attenuation [1]
73 and transcriptional activation of genes involved in ER protein folding and degradation [2, 3], the activation of
74 the UPR is required to overcome ER stress. The UPR is conserved from protozoa but exhibits a high degree
75 of complexity in metazoans [4]. The mammalian UPR programme regulates the expression of hundreds of
76 genes in response to signalling from the three sensor molecules ATF6 (activating transcription factor 6), IRE1
77 (inositol requiring enzyme 1), and PERK (double-stranded RNA-activated protein kinase (PKR)-like ER
78 kinase), all three of which are embedded within the ER membrane (Fig. 1). IRE1 is the most evolutionarily
79 conserved UPR sensor, and it is the sole sensor present in lower eukaryotes including *Saccharomyces*
80 *cerevisiae* and *Schizosaccharomyces pombe*. As part of the initial adaptive phase of the UPR, accumulation
81 of unfolded proteins within the ER lumen activates the three ER stress sensors which further initiates a cascade
82 of signalling events that aim to restore and protect cellular integrity [5, 6]. However, cellular dysfunction and
83 cell death often occurs when ER stress is chronically prolonged and protein load overwhelms the folding
84 capacity of the ER. Additionally, the three transducer proteins sense ER stress through lipid bilayer stress
85 (LBS), in a manner independent from their respective luminal domains [7-11]. The molecular mechanism of
86 IRE1 activation upon LBS has been recently demonstrated in budding yeast [7]. Various perturbations of lipid
87 metabolism have been shown to activate IRE1, including altered ratio of phosphatidylethanolamine (PE) and
88 phosphatidylcholine (PC) [10], elevated lipid saturation [12], elevated sterol levels [13], and inositol depletion
89 [9]. Hence, LBS was introduced as a collective term for atypical lipid compositions of the ER membrane that
90 can contribute to UPR activation [12].

91

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

105

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

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

114 activation of its unique ribonuclease domain. Subsequently, active Ire1 cleaves the intron of homologous to
115 Atf/Creb1 (*HAC1*) mRNA, which is translated into the transcription factor Hac1 that upregulates the expression
116 of hundreds of genes involved in various cellular processes to restore ER homeostasis [10, 19, 20]. In
117 mammals, IRE1 α and IRE1 β are the two IRE1 homologues of yeast Ire1 [21, 22], where IRE1 α is ubiquitously
118 expressed and therefore the main UPR mediator, while IRE1 β expression is limited to the epithelial cells of the
119 gastrointestinal tract [22, 23]. Conserved in higher organisms, IRE1 α is similarly activated and it splices the
120 pre-mRNA of the transcription factor X-box binding protein 1 (*XBP1*) [24, 25] (Fig. 1A). In addition to the
121 interaction between IRE1 α and the Sec61 translocon complex, the signal recognition particle (SRP) pathway
122 recruitment of *XBP1*^u to the Sec61 translocon is also required for efficient cleavage of *XBP1*^u [26, 27]. The
123 spliced *XBP1* mRNA is then translated into an active transcription factor that induces UPR target genes to
124 alleviate ER stress. In the event of prolonged ER stress, IRE1 α cleaves mRNAs localised to the ER membrane
125 by a mechanism termed as regulated IRE1-dependent decay of mRNA (RIDD) (Fig. 1A) [28, 29]. The activation
126 of RIDD further complements the UPR by reducing the incoming protein load on the ER under stress conditions.
127 The addition of two new classes of ER stress sensors, PERK and ATF6, has increased the complexity of the
128 ER stress sensing and response mechanisms in higher eukaryotes. Oligomerisation of PERK triggers
129 autophosphorylation and the phosphorylation of eukaryotic translation initiation factor 2 subunit alpha (eIF2 α)
130 during UPR^{LBS} and UPR^{PT} [11] (Fig. 1B). Phosphorylated eIF2 α (eIF2 α -P) attenuates general protein
131 translation while enhancing the translation of the activating transcription factor 4 (ATF4). Subsequently, ATF4
132 induces a subset of UPR genes including pro-apoptotic factor C/EBP-homologous protein (CHOP) and the
133 growth arrest and DNA damage-inducible protein (GADD34). GADD34 promotes the dephosphorylation of
134 eIF2 α -P to subsequently derepress translation. The transcription factor CHOP induces pro-apoptotic genes in
135 the presence of persistent stress such as prolonged ER stress [30]. In contrast to IRE1 α and PERK, disulfide-

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

144

145 *2.1. Sensing proteotoxic stress at the ER*

146 Protein glycosylation is a sophisticated post-translational modification that plays an important role in the folding,
147 stability, subcellular localisation and biological function of proteins residing or transiting through the secretory
148 pathway. Initially known to function as a natural antibiotic, tunicamycin inhibits *N*-linked glycosylation in the ER
149 of eukaryotic cells, thereby leading to the disruption of protein folding and the severe accumulation of unfolded
150 proteins within the ER [33-35]. Pioneer work to dissect the ER stress sensing mechanism pointed to the
151 accumulation of unfolded proteins in the ER lumen. In the late 80's, either the expression of unfolded influenza
152 haemagglutinin viral protein or tunicamycin treatment were shown to induce the expression of the ER-resident
153 molecular chaperones immunoglobulin heavy-chain-binding protein (BiP), 94 kDa glucose-regulated protein
154 (GRP94), and protein disulfide-isomerase (PDI) [36, 37]. ER molecular chaperones facilitate protein
155 translocation, folding, and quality control [20]. Subsequently, the missing link between ER stress and gene
156 regulation, including ER-resident molecular chaperones, was elucidated in *S. cerevisiae*. The accumulation of
157 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 IRE1 α /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 eIF2 α 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

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

183

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

224 leucine zipper (bZIP) transcription factor domain [43, 44]. The luminal domain of ATF6 bears no sequence
225 homology to either IRE1 or PERK, and its mechanism of activation is dependent on changes in subcellular
226 localization and intramembrane proteolysis [43, 67]. The luminal domain of ATF6 is essential and sufficient for
227 its activation under ER-stressed conditions, indicating that this domain senses ER stress signal within the ER
228 [68]. Subsequently, BiP has been shown to bind to the luminal domain of ATF6 and to regulate its activation
229 [69]. Binding of BiP retains ATF6 in the ER by hindering its Golgi localization signals (GLSs) while the
230 dissociation of BiP during ER stress promotes the transportation of ATF6 to the Golgi [69]. Despite sharing a
231 similar mode of regulation by BiP with IRE1 and PERK, a mutational study showed that BiP-ATF6 complex is
232 stable and BiP dissociation during ER stress occurs through an active mechanism rather than the competition
233 with unfolded proteins described above for IRE1 [70]. Unlike IRE1 and PERK, ATF6 exists as a monomer,
234 dimer and oligomer, with the higher order forms maintained during non-ER-stressed condition via inter- and
235 intramolecular disulfide bridges formed between the two conserved cysteine residues within its luminal domain
236 [32]. Mutation of both conserved cysteine residues that mediate oligomerisation failed to induce constitutive
237 trafficking of ATF6 to the Golgi apparatus, suggesting that monomeric ATF6 is not sufficient for its activation
238 [32]. Upon ER stress, a reduced form of monomeric ATF6 translocates to the Golgi apparatus where its amino-
239 terminal transcription factor domain is released by S1P and S2P-mediated proteolysis. The conversion to the
240 monomeric form of ATF6 seems to be important for its activation as oligomers are absent in the Golgi apparatus
241 and they are also poor substrates of S1P cleavage [32]. The reduction process is specific to ATF6, as another
242 ER membrane-bound transcription factor, LZIP, was not reduced upon ER stress [32]. In addition, the
243 glycosylation inhibitor tunicamycin reduced ATF6 similar to the reducing agent DTT, reflecting the correlation
244 between reduction and ATF6 activation [32]. This further suggests that the mechanism underlying the activation
245 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 (Ire1 Δ LD) 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 IRE1 α and PERK where LBS
267 was induced by abnormally elevated levels of saturated fatty acids within the biological membrane [11]. IRE1 α

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

282

283 Recently, ATF6 was reported to be specifically activated from LBS but through a divergent sensing mechanism
284 from those of IRE1 α and PERK [83]. ATF6 was shown to be sensitive to the accumulation of two sphingolipid
285 species, namely dihydrosphingosine (DHS) and dihydroceramide (DHC), while being non-responsive to
286 ceramides (Fig. 2C). Although both sphingolipid intermediates are normally in low abundance, the authors
287 demonstrated their levels are elevated upon ER stress. DHS and DHC accumulation activate ATF6 similarly
288 as proteotoxic stress (Fig. 1C). Single point mutations within the ATF6 transmembrane conserved motif were
289 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 $\Delta 9$ -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.

312

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

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

325

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

334

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

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

364

365 *3.1. Transcriptional and non-transcriptional UPR programme upon proteotoxic stress*

366 Each UPR transducer regulates its downstream transcription factor, and the combination of the three UPR
367 branches generates a variety of responses. These transcription factors also regulate an overlapping subset of
368 genes involved in both ER-specific and general cellular proteostasis pathways [109-112]. Among the hundreds
369 of upregulated genes in eukaryotes, a subset encodes ER chaperones, protein trafficking machinery, protein
370 quality control, lipid metabolism, and autophagy [8, 19, 20, 24, 25, 113]. These genes are rapidly and efficiently
371 transcribed by the presence of ER stress response element (ERSE) and UPR element (UPRE) within their
372 promoter regions. To increase specificity, certain genes contain unique self-regulatory loops to further promote
373 the robustness of the UPR in response to different cellular stresses [114]. For instance, transcriptional levels
374 of ERSE-bearing genes *GRP78* (BiP protein), *XBP1*, and *CHOP* are increased by thapsigargin-induced
375 depletion of ER stored calcium, which can be further self-amplified with higher concentration of thapsigargin.
376 On the other hand, UPRE-bearing genes ATF4, ATF6, IRE1, and PERK lack positive feedback loops upon
377 acute ER stress. In addition to temporary translation attenuation through eIF2 α phosphorylation, PERK

378 activates the nuclear factor-erythroid 2-related factor 2 (NRF2) to upregulate genes encoding antioxidant
379 proteins and detoxifying enzymes [115]. Both PERK and IRE1 independently induce the transcription factor
380 NF- κ B to potentially promote inflammation, immune responses, apoptosis and tumorigenesis [116, 117] (Fig.
381 1A).

382

383 ATF4-induced CHOP regulates the growth arrest and GADD34, a regulatory subunit of protein phosphatase
384 type 1 (PP1) [118]. GADD34, together with PP1C, dephosphorylates eIF2 α to resume translation of proteins
385 [119, 120] (Fig. 1B). Resumed protein translation in stressed cells will eventually increase the ER protein load,
386 further promoting ER stress-induced apoptosis [118, 121, 122]. The regulated IRE1-dependent decay (RIDD)
387 pathway complements the general transcriptional and translational regulation during chronic ER stress (Fig.
388 1A) [28]. IRE1 mediates the degradation of newly targeted mRNAs or existing mRNAs at the ER translocon
389 site to reduce overall ER protein load [123]. This activity results in the termination of protein synthesis at the
390 ER and thus reduces protein overload within the ER [28, 29, 123]. In addition to its pro-survival properties,
391 IRE1 additionally exhibits pro-apoptotic properties by phosphorylating c-Jun amino-terminal kinase (JNK)
392 during chronic ER stress [124]. Activated JNK phosphorylates and suppresses the anti-apoptotic activity of the
393 B-cell CLL/lymphoma 2 (BCL2) family of proteins and activate pro-apoptotic BCL2 homology domain 3 (BH3)-
394 only proteins [125-127]. Despite its recognized pro-apoptotic role, JNK signalling can upregulate autophagy to
395 promote cell survival [128]. Central to the pro-apoptotic role of PERK, CHOP induces a transcriptional
396 programme that is similar to JNK-induced apoptosis signalling pathway. Additionally, CHOP activates the
397 death receptor 5 (DR5) by sensitising cells to different conditions that trigger ER stress [118, 129]. To further
398 amplify the transcriptional regulatory network of the UPR programme, ATF6(N) upregulates *CHOP* and *XBP1*
399 [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

466 identified to either inhibit UPR pro-survival effects or to attenuate ER stress levels. For instance, the compound
467 GSK2656157 has been identified to inhibit the kinase activity of PERK which proved to be promising in
468 targeting pancreatic adenocarcinoma and multiple myeloma in preclinical models [142]. However, it failed to
469 progress further due to undesirable side effects that caused pancreatic β -cell loss and diabetes [143], similar
470 to other inhibitors of PERK kinase activity. IRE1 α , XBP1, and PERK inhibitors could be promising in treating
471 diseases but their potential long-term side effects on the function of the pancreas, the immune system, and
472 the liver need to be carefully assessed. In particular, the liver plays a critical role in cholesterol and lipid
473 metabolism in addition of drug detoxification through the Ire1 α /XBP1 axes [144-146]. Consequently, the UPR
474 programme may be too broad to be inhibited from the upstream effectors. Thus, specific inhibition of a subset
475 of downstream targets that are part of a specific UPR transcriptional programme may yield better success and
476 potentially minimise undesirable long-term side effects. In order to identify such targets, substantial efforts
477 should be made to characterise stress-specific UPR transcriptional programmes in the near future.

478

479 **ACKNOWLEDGEMENTS**

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

484

485 **FIGURE LEGENDS**

486 **Figure 1.** Activation of the unfolded protein response (UPR). (A) In mammals, the accumulation of unfolded
487 proteins or lipid bilayer stress (LBS) result in ER stress. (A) Upon sensing stress, IRE1 α dimerisation or higher

488 oligomers from monomer, followed by trans-autophosphorylation. Subsequently, IRE1 α splices *XBP1* mRNA,
489 resulting in the translation of the transcription factor XBP1 which regulates a subset of UPR genes. Prolonged
490 ER stress activates the regulated IRE1-dependent decay (RIDD), cleaving a subset of mRNA to decrease
491 mRNA load. Both JNK and NF- κ B are activated by IRE1 α to initiate the transcription of proinflammatory genes.
492 (B) PERK oligomerises and phosphorylates itself and eIF2 α . eIF2 α -P attenuates general protein translation
493 while activating the translation of the transcription factor ATF4. Subsequently, ATF4 activates a subset of UPR
494 genes including pro-apoptotic factor CHOP and GADD34. GADD34 promotes the dephosphorylation of eIF2 α ,
495 resuming translation. (C) ATF6 is transported to the Golgi apparatus where the N-terminal transcription factor
496 [ATF6(N)] is released by intramembrane proteolysis. ATF6(N) regulates a subset of UPR genes.

497

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

506

507 **Figure 3.** The deployed UPR programme is crafted to stress-specific-induced ER stress. The three ER stress
508 sensors IRE1, PERK, and ATF6 activate their conserved linear pathways resulting in activation and inhibition
509 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

512 **REFERENCES**

- 513 [1] H.P. Harding, Y. Zhang, D. Ron, Protein translation and folding are coupled by an endoplasmic-reticulum-
514 resident kinase, *Nature*, 397 (1999) 271-274.
- 515 [2] M.J. Gething, J. Sambrook, Protein folding in the cell, *Nature*, 355 (1992) 33-45.
- 516 [3] K. Mori, Tripartite management of unfolded proteins in the endoplasmic reticulum, *Cell*, 101 (2000) 451-
517 454.
- 518 [4] J. Hollien, Evolution of the unfolded protein response, *Biochim Biophys Acta*, 1833 (2013) 2458-2463.
- 519 [5] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, *Nat Rev Mol*
520 *Cell Biol*, 8 (2007) 519-529.
- 521 [6] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, *Science*,
522 334 (2011) 1081-1086.
- 523 [7] K. Halbleib, K. Pesek, R. Covino, H.F. Hofbauer, D. Wunnicke, I. Hanelt, G. Hummer, R. Ernst, Activation
524 of the Unfolded Protein Response by Lipid Bilayer Stress, *Mol Cell*, 67 (2017) 673-684 e678.
- 525 [8] J.H. Koh, L. Wang, C. Beaudoin-Chabot, G. Thibault, Lipid bilayer stress-activated IRE-1 modulates
526 autophagy during endoplasmic reticulum stress, *J Cell Sci*, 131 (2018).
- 527 [9] T. Promlek, Y. Ishiwata-Kimata, M. Shido, M. Sakuramoto, K. Kohno, Y. Kimata, Membrane aberrancy and
528 unfolded proteins activate the endoplasmic reticulum stress sensor Ire1 in different ways, *Mol Biol Cell*, 22
529 (2011) 3520-3532.
- 530 [10] G. Thibault, G. Shui, W. Kim, G.C. McAlister, N. Ismail, S.P. Gygi, M.R. Wenk, D.T. Ng, The membrane
531 stress response buffers lethal effects of lipid disequilibrium by reprogramming the protein homeostasis network,
532 *Mol Cell*, 48 (2012) 16-27.
- 533 [11] R. Volmer, K. van der Ploeg, D. Ron, Membrane lipid saturation activates endoplasmic reticulum unfolded
534 protein response transducers through their transmembrane domains, *Proc Natl Acad Sci U S A*, 110 (2013)
535 4628-4633.
- 536 [12] M.A. Surma, C. Klose, D. Peng, M. Shales, C. Mrejen, A. Stefanko, H. Braberg, D.E. Gordon, D. Vorkel,
537 C.S. Ejsing, R. Farese, Jr., K. Simons, N.J. Krogan, R. Ernst, A lipid E-MAP identifies Ubx2 as a critical
538 regulator of lipid saturation and lipid bilayer stress, *Mol Cell*, 51 (2013) 519-530.
- 539 [13] L. Pineau, J. Colas, S. Dupont, L. Beney, P. Fleurat-Lessard, J.M. Berjeaud, T. Berges, T. Ferreira, Lipid-
540 induced ER stress: synergistic effects of sterols and saturated fatty acids, *Traffic*, 10 (2009) 673-690.
- 541 [14] T. Kawahara, H. Yanagi, T. Yura, K. Mori, Endoplasmic reticulum stress-induced mRNA splicing permits
542 synthesis of transcription factor Hac1p/Ern4p that activates the unfolded protein response, *Mol Biol Cell*, 8
543 (1997) 1845-1862.
- 544 [15] J.S. Cox, C.E. Shamu, P. Walter, Transcriptional induction of genes encoding endoplasmic reticulum
545 resident proteins requires a transmembrane protein kinase, *Cell*, 73 (1993) 1197-1206.
- 546 [16] C.E. Shamu, P. Walter, Oligomerization and phosphorylation of the Ire1p kinase during intracellular
547 signaling from the endoplasmic reticulum to the nucleus, *EMBO J*, 15 (1996) 3028-3039.
- 548 [17] Y. Kimata, Y. Ishiwata-Kimata, T. Ito, A. Hirata, T. Suzuki, D. Oikawa, M. Takeuchi, K. Kohno, Two
549 regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins,
550 *J Cell Biol*, 179 (2007) 75-86.
- 551 [18] H. Li, A.V. Korennykh, S.L. Behrman, P. Walter, Mammalian endoplasmic reticulum stress sensor IRE1
552 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. Calton, 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 malformed 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.

596 [38] K. Mori, W. Ma, M.J. Gething, J. Sambrook, A transmembrane protein with a cdc2+/CDC28-related kinase
597 activity is required for signaling from the ER to the nucleus, *Cell*, 74 (1993) 743-756.

598 [39] J.S. Cox, P. Walter, A novel mechanism for regulating activity of a transcription factor that controls the
599 unfolded protein response, *Cell*, 87 (1996) 391-404.

600 [40] C.R. Prostko, M.A. Brostrom, C.O. Brostrom, Reversible phosphorylation of eukaryotic initiation factor 2
601 alpha in response to endoplasmic reticular signaling, *Mol Cell Biochem*, 127-128 (1993) 255-265.

602 [41] W.L. Wong, M.A. Brostrom, G. Kuznetsov, D. Gmitter-Yellen, C.O. Brostrom, Inhibition of protein synthesis
603 and early protein processing by thapsigargin in cultured cells, *Biochem J*, 289 (Pt 1) (1993) 71-79.

604 [42] C.O. Brostrom, M.A. Brostrom, Regulation of translational initiation during cellular responses to stress,
605 *Prog Nucleic Acid Res Mol Biol*, 58 (1998) 79-125.

606 [43] K. Haze, H. Yoshida, H. Yanagi, T. Yura, K. Mori, Mammalian transcription factor ATF6 is synthesized as
607 a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress, *Mol Biol*
608 *Cell*, 10 (1999) 3787-3799.

609 [44] K. Haze, T. Okada, H. Yoshida, H. Yanagi, T. Yura, M. Negishi, K. Mori, Identification of the G13 (cAMP-
610 response-element-binding protein-related protein) gene product related to activating transcription factor 6 as
611 a transcriptional activator of the mammalian unfolded protein response, *Biochem J*, 355 (2001) 19-28.

612 [45] A. Bertolotti, Y. Zhang, L.M. Hendershot, H.P. Harding, D. Ron, Dynamic interaction of BiP and ER stress
613 transducers in the unfolded-protein response, *Nat Cell Biol*, 2 (2000) 326-332.

614 [46] K. Okamura, Y. Kimata, H. Higashio, A. Tsuru, K. Kohno, Dissociation of Kar2p/BiP from an ER sensory
615 molecule, Ire1p, triggers the unfolded protein response in yeast, *Biochem Biophys Res Commun*, 279 (2000)
616 445-450.

617 [47] Y. Kimata, D. Oikawa, Y. Shimizu, Y. Ishiwata-Kimata, K. Kohno, A role for BiP as an adjustor for the
618 endoplasmic reticulum stress-sensing protein Ire1, *J Cell Biol*, 167 (2004) 445-456.

619 [48] M. Carrara, F. Prischi, P.R. Nowak, M.C. Kopp, M.M. Ali, Noncanonical binding of BiP ATPase domain to
620 Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling, *Elife*, 4 (2015).

621 [49] M.C. Kopp, P.R. Nowak, N. Larburu, C.J. Adams, M.M. Ali, In vitro FRET analysis of IRE1 and BiP
622 association and dissociation upon endoplasmic reticulum stress, *Elife*, 7 (2018).

623 [50] J.J. Credle, J.S. Finer-Moore, F.R. Papa, R.M. Stroud, P. Walter, On the mechanism of sensing unfolded
624 protein in the endoplasmic reticulum, *Proc Natl Acad Sci U S A*, 102 (2005) 18773-18784.

625 [51] D. Oikawa, Y. Kimata, K. Kohno, Self-association and BiP dissociation are not sufficient for activation of
626 the ER stress sensor Ire1, *J Cell Sci*, 120 (2007) 1681-1688.

627 [52] B.M. Gardner, P. Walter, Unfolded proteins are Ire1-activating ligands that directly induce the unfolded
628 protein response, *Science*, 333 (2011) 1891-1894.

629 [53] G.E. Karagoz, D. Acosta-Alvear, H.T. Nguyen, C.P. Lee, F. Chu, P. Walter, An unfolded protein-induced
630 conformational switch activates mammalian IRE1, *Elife*, 6 (2017).

631 [54] K. Ma, K.M. Vattam, R.C. Wek, Dimerization and release of molecular chaperone inhibition facilitate
632 activation of eukaryotic initiation factor-2 kinase in response to endoplasmic reticulum stress, *J Biol Chem*, 277
633 (2002) 18728-18735.

634 [55] A.A. Welihinda, R.J. Kaufman, The unfolded protein response pathway in *Saccharomyces cerevisiae*.
635 Oligomerization and trans-phosphorylation of Ire1p (Ern1p) are required for kinase activation, *J Biol Chem*,
636 271 (1996) 18181-18187.

637 [56] C.Y. Liu, M. Schroder, R.J. Kaufman, Ligand-independent dimerization activates the stress response
638 kinases IRE1 and PERK in the lumen of the endoplasmic reticulum, *J Biol Chem*, 275 (2000) 24881-24885.

639 [57] M.M. Ali, T. Bagratuni, E.L. Davenport, P.R. Nowak, M.C. Silva-Santisteban, A. Hardcastle, C. McAndrews,
640 M.G. Rowlands, G.J. Morgan, W. Aherne, I. Collins, F.E. Davies, L.H. Pearl, Structure of the Ire1
641 autophosphorylation complex and implications for the unfolded protein response, *EMBO J*, 30 (2011) 894-905.
642 [58] F. Prischi, P.R. Nowak, M. Carrara, M.M. Ali, Phosphoregulation of Ire1 RNase splicing activity, *Nat*
643 *Commun*, 5 (2014) 3554.
644 [59] M. Carrara, F. Prischi, P.R. Nowak, M.M. Ali, Crystal structures reveal transient PERK luminal domain
645 tetramerization in endoplasmic reticulum stress signaling, *EMBO J*, 34 (2015) 1589-1600.
646 [60] P. Wang, J. Li, J. Tao, B. Sha, The luminal domain of the ER stress sensor protein PERK binds misfolded
647 proteins and thereby triggers PERK oligomerization, *J Biol Chem*, 293 (2018) 4110-4121.
648 [61] B. Sha, S. Lee, D.M. Cyr, The crystal structure of the peptide-binding fragment from the yeast Hsp40
649 protein Sis1, *Structure*, 8 (2000) 799-807.
650 [62] J. Li, X. Qian, B. Sha, The crystal structure of the yeast Hsp40 Ydj1 complexed with its peptide substrate,
651 *Structure*, 11 (2003) 1475-1483.
652 [63] A.L. Horwich, W.A. Fenton, E. Chapman, G.W. Farr, Two families of chaperonin: physiology and
653 mechanism, *Annu Rev Cell Dev Biol*, 23 (2007) 115-145.
654 [64] N. Jaya, V. Garcia, E. Vierling, Substrate binding site flexibility of the small heat shock protein molecular
655 chaperones, *Proc Natl Acad Sci U S A*, 106 (2009) 15604-15609.
656 [65] M. Taipale, D.F. Jarosz, S. Lindquist, HSP90 at the hub of protein homeostasis: emerging mechanistic
657 insights, *Nat Rev Mol Cell Biol*, 11 (2010) 515-528.
658 [66] D.R. Southworth, D.A. Agard, Client-loading conformation of the Hsp90 molecular chaperone revealed in
659 the cryo-EM structure of the human Hsp90:Hop complex, *Mol Cell*, 42 (2011) 771-781.
660 [67] H. Yoshida, K. Haze, H. Yanagi, T. Yura, K. Mori, Identification of the cis-acting endoplasmic reticulum
661 stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins.
662 Involvement of basic leucine zipper transcription factors, *J Biol Chem*, 273 (1998) 33741-33749.
663 [68] X. Chen, J. Shen, R. Prywes, The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and
664 causes translocation of ATF6 from the ER to the Golgi, *J Biol Chem*, 277 (2002) 13045-13052.
665 [69] J. Shen, X. Chen, L. Hendershot, R. Prywes, ER stress regulation of ATF6 localization by dissociation of
666 BiP/GRP78 binding and unmasking of Golgi localization signals, *Dev Cell*, 3 (2002) 99-111.
667 [70] J. Shen, E.L. Snapp, J. Lippincott-Schwartz, R. Prywes, Stable binding of ATF6 to BiP in the endoplasmic
668 reticulum stress response, *Mol Cell Biol*, 25 (2005) 921-932.
669 [71] S. Nadanaka, H. Yoshida, K. Mori, Reduction of disulfide bridges in the luminal domain of ATF6 in
670 response to glucose starvation, *Cell Struct Funct*, 31 (2006) 127-134.
671 [72] Y. Sato, S. Nadanaka, T. Okada, K. Okawa, K. Mori, Luminal domain of ATF6 alone is sufficient for sensing
672 endoplasmic reticulum stress and subsequent transport to the Golgi apparatus, *Cell Struct Funct*, 36 (2011)
673 35-47.
674 [73] S.A. Jesch, X. Zhao, M.T. Wells, S.A. Henry, Genome-wide analysis reveals inositol, not choline, as the
675 major effector of Ino2p-Ino4p and unfolded protein response target gene expression in yeast, *J Biol Chem*,
676 280 (2005) 9106-9118.
677 [74] S.A. Jesch, P. Liu, X. Zhao, M.T. Wells, S.A. Henry, Multiple endoplasmic reticulum-to-nucleus signaling
678 pathways coordinate phospholipid metabolism with gene expression by distinct mechanisms, *J Biol Chem*,
679 281 (2006) 24070-24083.
680 [75] S. Fu, L. Yang, P. Li, O. Hofmann, L. Dicker, W. Hide, X. Lin, S.M. Watkins, A.R. Ivanov, G.S. Hotamisligil,
681 Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity,
682 *Nature*, 473 (2011) 528-531.

683 [76] W. Cui, J. Ma, X. Wang, W. Yang, J. Zhang, Q. Ji, Free fatty acid induces endoplasmic reticulum stress
684 and apoptosis of beta-cells by Ca²⁺/calpain-2 pathways, *PLoS One*, 8 (2013) e59921.

685 [77] Q. Xie, V.I. Khaoustov, C.C. Chung, J. Sohn, B. Krishnan, D.E. Lewis, B. Yoffe, Effect of
686 tauroursodeoxycholic acid on endoplasmic reticulum stress-induced caspase-12 activation, *Hepatology*, 36
687 (2002) 592-601.

688 [78] C.R. Brown, L.Q. Hong-Brown, J. Biwersi, A.S. Verkman, W.J. Welch, Chemical chaperones correct the
689 mutant phenotype of the delta F508 cystic fibrosis transmembrane conductance regulator protein, *Cell Stress*
690 *Chaperones*, 1 (1996) 117-125.

691 [79] W.J. Welch, C.R. Brown, Influence of molecular and chemical chaperones on protein folding, *Cell Stress*
692 *Chaperones*, 1 (1996) 109-115.

693 [80] T. Kolter, M. Wendeler, Chemical chaperones--a new concept in drug research, *Chembiochem*, 4 (2003)
694 260-264.

695 [81] U. Ozcan, E. Yilmaz, L. Ozcan, M. Furuhashi, E. Vaillancourt, R.O. Smith, C.Z. Gorgun, G.S. Hotamisligil,
696 Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes,
697 *Science*, 313 (2006) 1137-1140.

698 [82] T.W. Loo, D.M. Clarke, Chemical and pharmacological chaperones as new therapeutic agents, *Expert*
699 *Rev Mol Med*, 9 (2007) 1-18.

700 [83] A.B. Tam, L.S. Roberts, V. Chandra, I.G. Rivera, D.K. Nomura, D.J. Forbes, M. Niwa, The UPR Activator
701 ATF6 Responds to Proteotoxic and Lipotoxic Stress by Distinct Mechanisms, *Dev Cell*, 46 (2018) 327-343
702 e327.

703 [84] N. Kono, N. Amin-Wetzel, D. Ron, Generic membrane-spanning features endow IRE1alpha with
704 responsiveness to membrane aberrancy, *Mol Biol Cell*, 28 (2017) 2318-2332.

705 [85] F.X. Contreras, A.M. Ernst, P. Haberkant, P. Bjorkholm, E. Lindahl, B. Gonen, C. Tischer, A. Elofsson, G.
706 von Heijne, C. Thiele, R. Pepperkok, F. Wieland, B. Brugger, Molecular recognition of a single sphingolipid
707 species by a protein's transmembrane domain, *Nature*, 481 (2012) 525-529.

708 [86] J.E. Stucky, V.M. McDonough, C.E. Martin, Isolation and characterization of OLE1, a gene affecting fatty
709 acid desaturation from *Saccharomyces cerevisiae*, *J Biol Chem*, 264 (1989) 16537-16544.

710 [87] S. Zhang, Y. Skalsky, D.J. Garfinkel, MGA2 or SPT23 is required for transcription of the delta9 fatty acid
711 desaturase gene, OLE1, and nuclear membrane integrity in *Saccharomyces cerevisiae*, *Genetics*, 151 (1999)
712 473-483.

713 [88] T. Hoppe, K. Matuschewski, M. Rape, S. Schlenker, H.D. Ulrich, S. Jentsch, Activation of a membrane-
714 bound transcription factor by regulated ubiquitin/proteasome-dependent processing, *Cell*, 102 (2000) 577-586.

715 [89] M. Rape, T. Hoppe, I. Gorr, M. Kalocay, H. Richly, S. Jentsch, Mobilization of processed, membrane-
716 tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone, *Cell*, 107 (2001)
717 667-677.

718 [90] N. Shcherbik, D.S. Haines, Cdc48p(Npl4p/Ufd1p) binds and segregates membrane-anchored/tethered
719 complexes via a polyubiquitin signal present on the anchors, *Mol Cell*, 25 (2007) 385-397.

720 [91] R. Covino, S. Ballweg, C. Stordeur, J.B. Michaelis, K. Puth, F. Wernig, A. Bahrami, A.M. Ernst, G. Hummer,
721 R. Ernst, A Eukaryotic Sensor for Membrane Lipid Saturation, *Mol Cell*, 63 (2016) 49-59.

722 [92] J.L. Goldstein, R.A. DeBose-Boyd, M.S. Brown, Protein sensors for membrane sterols, *Cell*, 124 (2006)
723 35-46.

724 [93] P.J. Espenshade, A.L. Hughes, Regulation of sterol synthesis in eukaryotes, *Annu Rev Genet*, 41 (2007)
725 401-427.

726 [94] J. Ye, R.B. Rawson, R. Komuro, X. Chen, U.P. Dave, R. Prywes, M.S. Brown, J.L. Goldstein, ER stress
727 induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs, *Mol Cell*, 6 (2000)
728 1355-1364.

729 [95] M.S. Brown, J.L. Goldstein, The SREBP pathway: regulation of cholesterol metabolism by proteolysis of
730 a membrane-bound transcription factor, *Cell*, 89 (1997) 331-340.

731 [96] C.L. Theesfeld, D. Pourmand, T. Davis, R.M. Garza, R.Y. Hampton, The sterol-sensing domain (SSD)
732 directly mediates signal-regulated endoplasmic reticulum-associated degradation (ERAD) of 3-hydroxy-3-
733 methylglutaryl (HMG)-CoA reductase isozyme Hmg2, *J Biol Chem*, 286 (2011) 26298-26307.

734 [97] K.A. Moore, J. Hollien, The unfolded protein response in secretory cell function, *Annu Rev Genet*, 46
735 (2012) 165-183.

736 [98] A.H. Lee, G.C. Chu, N.N. Iwakoshi, L.H. Glimcher, XBP-1 is required for biogenesis of cellular secretory
737 machinery of exocrine glands, *EMBO J*, 24 (2005) 4368-4380.

738 [99] D. Acosta-Alvear, Y. Zhou, A. Blais, M. Tsikitis, N.H. Lents, C. Arias, C.J. Lennon, Y. Kluger, B.D. Dynlacht,
739 XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks, *Mol Cell*, 27 (2007)
740 53-66.

741 [100] H. Ilieva, M. Polymenidou, D.W. Cleveland, Non-cell autonomous toxicity in neurodegenerative disorders:
742 ALS and beyond, *J Cell Biol*, 187 (2009) 761-772.

743 [101] J.J. Rodvold, K.T. Chiu, N. Hiramatsu, J.K. Nussbacher, V. Galimberti, N.R. Mahadevan, K. Willert, J.H.
744 Lin, M. Zanetti, Intercellular transmission of the unfolded protein response promotes survival and drug
745 resistance in cancer cells, *Sci Signal*, 10 (2017).

746 [102] R.C. Taylor, A. Dillin, XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity, *Cell*,
747 153 (2013) 1435-1447.

748 [103] J.R. Daniele, R. Higuchi-Sanabria, V. Ramachandran, M. Sanchez, J. Durieux, S.U. Tronnes, J.W. Paul,
749 D.J. Esping, S. Monshietehadi, M.G. Metcalf, A. Dillin, A non-canonical arm of UPR^{ER} mediates
750 longevity through ER remodeling and lipophagy, *bioRxiv*, (2018) 471177.

751 [104] X. Shen, R.E. Ellis, K. Sakaki, R.J. Kaufman, Genetic interactions due to constitutive and inducible gene
752 regulation mediated by the unfolded protein response in *C. elegans*, *PLoS Genet*, 1 (2005) e37.

753 [105] M.C. Jonikas, S.R. Collins, V. Denic, E. Oh, E.M. Quan, V. Schmid, J. Weibezahn, B. Schwappach, P.
754 Walter, J.S. Weissman, M. Schuldiner, Comprehensive characterization of genes required for protein folding
755 in the endoplasmic reticulum, *Science*, 323 (2009) 1693-1697.

756 [106] P. Lajoie, R.D. Moir, I.M. Willis, E.L. Snapp, Kar2p availability defines distinct forms of endoplasmic
757 reticulum stress in living cells, *Mol Biol Cell*, 23 (2012) 955-964.

758 [107] N.S. Hou, A. Gutschmidt, D.Y. Choi, K. Pather, X. Shi, J.L. Watts, T. Hoppe, S. Taubert, Activation of the
759 endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis in vivo,
760 *Proc Natl Acad Sci U S A*, 111 (2014) E2271-2280.

761 [108] C. Hetz, E. Chevet, H.P. Harding, Targeting the unfolded protein response in disease, *Nat Rev Drug*
762 *Discov*, 12 (2013) 703-719.

763 [109] T. Okada, H. Yoshida, R. Akazawa, M. Negishi, K. Mori, Distinct roles of activating transcription factor 6
764 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in
765 transcription during the mammalian unfolded protein response, *Biochem J*, 366 (2002) 585-594.

766 [110] A.H. Lee, N.N. Iwakoshi, L.H. Glimcher, XBP-1 regulates a subset of endoplasmic reticulum resident
767 chaperone genes in the unfolded protein response, *Mol Cell Biol*, 23 (2003) 7448-7459.

768 [111] K. Yamamoto, H. Yoshida, K. Kokame, R.J. Kaufman, K. Mori, Differential contributions of ATF6 and
769 XBP1 to the activation of endoplasmic reticulum stress-responsive cis-acting elements ERSE, UPRE and
770 ERSE-II, *J Biochem*, 136 (2004) 343-350.

771 [112] Y. Adachi, K. Yamamoto, T. Okada, H. Yoshida, A. Harada, K. Mori, ATF6 is a transcription factor
772 specializing in the regulation of quality control proteins in the endoplasmic reticulum, *Cell Struct Funct*, 33
773 (2008) 75-89.

774 [113] J.S. Shen, X. Chen, L. Hendershot, R. Prywes, ER stress regulation of ATF6 localization by dissociation
775 of BiP/GRP78 binding and unmasking of golgi localization signals, *Developmental Cell*, 3 (2002) 99-111.

776 [114] S. Takayanagi, R. Fukuda, Y. Takeuchi, S. Tsukada, K. Yoshida, Gene regulatory network of unfolded
777 protein response genes in endoplasmic reticulum stress, *Cell Stress Chaperones*, 18 (2013) 11-23.

778 [115] S.B. Cullinan, J.A. Diehl, PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell
779 survival following endoplasmic reticulum stress, *J Biol Chem*, 279 (2004) 20108-20117.

780 [116] M.S. Hayden, S. Ghosh, Signaling to NF-kappaB, *Genes Dev*, 18 (2004) 2195-2224.

781 [117] A.B. Tam, E.L. Mercado, A. Hoffmann, M. Niwa, ER stress activates NF-kappaB by integrating functions
782 of basal IKK activity, IRE1 and PERK, *PLoS One*, 7 (2012) e45078.

783 [118] S.J. Marciniak, C.Y. Yun, S. Oyadomari, I. Novoa, Y. Zhang, R. Jungreis, K. Nagata, H.P. Harding, D.
784 Ron, CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum,
785 *Genes Dev*, 18 (2004) 3066-3077.

786 [119] I. Novoa, H. Zeng, H.P. Harding, D. Ron, Feedback inhibition of the unfolded protein response by
787 GADD34-mediated dephosphorylation of eIF2alpha, *J Cell Biol*, 153 (2001) 1011-1022.

788 [120] Y. Ma, L.M. Hendershot, Delineation of a negative feedback regulatory loop that controls protein
789 translation during endoplasmic reticulum stress, *J Biol Chem*, 278 (2003) 34864-34873.

790 [121] M.H. Brush, D.C. Weiser, S. Shenolikar, Growth arrest and DNA damage-inducible protein GADD34
791 targets protein phosphatase 1 alpha to the endoplasmic reticulum and promotes dephosphorylation of the
792 alpha subunit of eukaryotic translation initiation factor 2, *Mol Cell Biol*, 23 (2003) 1292-1303.

793 [122] D.E. Feldman, V. Chauhan, A.C. Koong, The unfolded protein response: a novel component of the
794 hypoxic stress response in tumors, *Mol Cancer Res*, 3 (2005) 597-605.

795 [123] D. Acosta-Alvear, G.E. Karagoz, F. Frohlich, H. Li, T.C. Walther, P. Walter, The unfolded protein
796 response and endoplasmic reticulum protein targeting machineries converge on the stress sensor IRE1, *Elife*,
797 7 (2018).

798 [124] F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H.P. Harding, D. Ron, Coupling of stress in the ER
799 to activation of JNK protein kinases by transmembrane protein kinase IRE1, *Science*, 287 (2000) 664-666.

800 [125] R.J. Davis, Signal transduction by the JNK group of MAP kinases, *Cell*, 103 (2000) 239-252.

801 [126] K. Lei, R.J. Davis, JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-
802 dependent apoptosis, *Proc Natl Acad Sci U S A*, 100 (2003) 2432-2437.

803 [127] M.C. Bassik, L. Scorrano, S.A. Oakes, T. Pozzan, S.J. Korsmeyer, Phosphorylation of BCL-2 regulates
804 ER Ca²⁺ homeostasis and apoptosis, *EMBO J*, 23 (2004) 1207-1216.

805 [128] Y. Wei, S. Pattingre, S. Sinha, M. Bassik, B. Levine, JNK1-mediated phosphorylation of Bcl-2 regulates
806 starvation-induced autophagy, *Mol Cell*, 30 (2008) 678-688.

807 [129] H. Yamaguchi, H.G. Wang, CHOP is involved in endoplasmic reticulum stress-induced apoptosis by
808 enhancing DR5 expression in human carcinoma cells, *J Biol Chem*, 279 (2004) 45495-45502.

809 [130] H. Yoshida, T. Okada, K. Haze, H. Yanagi, T. Yura, M. Negishi, K. Mori, ATF6 activated by proteolysis
810 binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded
811 protein response, *Mol Cell Biol*, 20 (2000) 6755-6767.

812 [131] K. Yamamoto, T. Sato, T. Matsui, M. Sato, T. Okada, H. Yoshida, A. Harada, K. Mori, Transcriptional
813 induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha
814 and XBP1, *Dev Cell*, 13 (2007) 365-376.

815 [132] B. Feng, P.M. Yao, Y. Li, C.M. Devlin, D. Zhang, H.P. Harding, M. Sweeney, J.X. Rong, G. Kuriakose,
816 E.A. Fisher, A.R. Marks, D. Ron, I. Tabas, The endoplasmic reticulum is the site of cholesterol-induced
817 cytotoxicity in macrophages, *Nat Cell Biol*, 5 (2003) 781-792.

818 [133] D.A. Cunha, P. Hekerman, L. Ladriere, A. Bazarra-Castro, F. Ortis, M.C. Wakeham, F. Moore, J.
819 Rasschaert, A.K. Cardozo, E. Bellomo, L. Overbergh, C. Mathieu, R. Lupi, T. Hai, A. Herchuelz, P. Marchetti,
820 G.A. Rutter, D.L. Eizirik, M. Cnop, Initiation and execution of lipotoxic ER stress in pancreatic beta-cells, *J Cell*
821 *Sci*, 121 (2008) 2308-2318.

822 [134] Y. Chen, Z. Wu, S. Zhao, R. Xiang, Chemical chaperones reduce ER stress and adipose tissue
823 inflammation in high fat diet-induced mouse model of obesity, *Sci Rep*, 6 (2016) 27486.

824 [135] M. Piccolis, L.M. Bond, M. Kampmann, P. Pulimeno, C. Chitraju, C.B.K. Jayson, L.P. Vaites, S. Boland,
825 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.,
826 Probing the Global Cellular Responses to Lipotoxicity Caused by Saturated Fatty Acids, *Mol Cell*, 74 (2019)
827 32-44 e38.

828 [136] R. Sriburi, S. Jackowski, K. Mori, J.W. Brewer, XBP1: a link between the unfolded protein response, lipid
829 biosynthesis, and biogenesis of the endoplasmic reticulum, *J Cell Biol*, 167 (2004) 35-41.

830 [137] S. Oyadomari, H.P. Harding, Y. Zhang, M. Oyadomari, D. Ron, Dephosphorylation of translation initiation
831 factor 2alpha enhances glucose tolerance and attenuates hepatosteatosis in mice, *Cell metabolism*, 7 (2008)
832 520-532.

833 [138] P. Puri, R.A. Baillie, M.M. Wiest, F. Mirshahi, J. Choudhury, O. Cheung, C. Sargeant, M.J. Contos, A.J.
834 Sanyal, A lipidomic analysis of nonalcoholic fatty liver disease, *Hepatology*, 46 (2007) 1081-1090.

835 [139] D.G. Tiniakos, M.B. Vos, E.M. Brunt, Nonalcoholic fatty liver disease: pathology and pathogenesis, *Annu*
836 *Rev Pathol*, 5 (2010) 145-171.

837 [140] B.M. Arendt, D.W. Ma, B. Simons, S.A. Noureldin, G. Therapondos, M. Guindi, M. Sherman, J.P. Allard,
838 Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine
839 to phosphatidylethanolamine, *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition*
840 *et metabolisme*, 38 (2013) 334-340.

841 [141] I. Doycheva, K.D. Watt, G. Rifai, R. Abou Mrad, R. Lopez, N.N. Zein, W.D. Carey, N. Alkhouri, Increasing
842 Burden of Chronic Liver Disease Among Adolescents and Young Adults in the USA: A Silent Epidemic, *Dig*
843 *Dis Sci*, 62 (2017) 1373-1380.

844 [142] C. Atkins, Q. Liu, E. Minthorn, S.Y. Zhang, D.J. Figueroa, K. Moss, T.B. Stanley, B. Sanders, A. Goetz,
845 N. Gaul, A.E. Choudhry, H. Alsaid, B.M. Jucker, J.M. Axten, R. Kumar, Characterization of a novel PERK
846 kinase inhibitor with antitumor and antiangiogenic activity, *Cancer Res*, 73 (2013) 1993-2002.

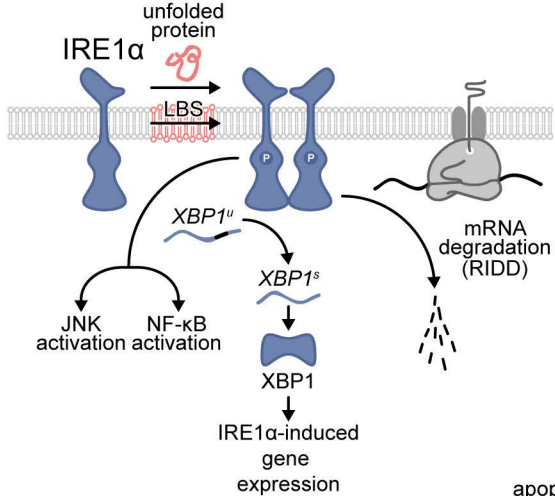
847 [143] S.A. Oakes, Endoplasmic reticulum proteostasis: a key checkpoint in cancer, *Am J Physiol Cell Physiol*,
848 312 (2017) C93-C102.

849 [144] J.S. So, K.Y. Hur, M. Tarrio, V. Ruda, M. Frank-Kamenetsky, K. Fitzgerald, V. Koteliansky, A.H. Lichtman,
850 T. Iwawaki, L.H. Glimcher, A.H. Lee, Silencing of lipid metabolism genes through IRE1alpha-mediated mRNA
851 decay lowers plasma lipids in mice, *Cell metabolism*, 16 (2012) 487-499.

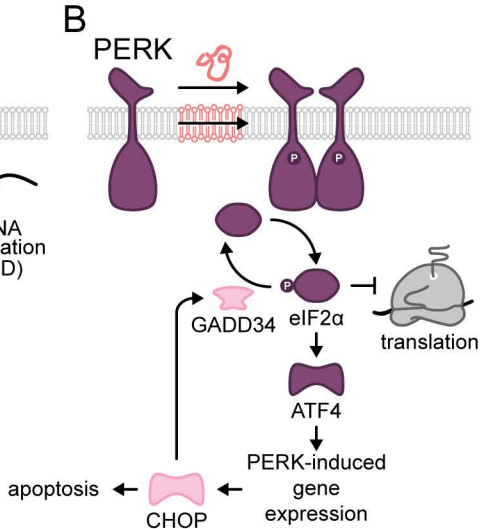
852 [145] S. Fu, S.M. Watkins, G.S. Hotamisligil, The role of endoplasmic reticulum in hepatic lipid homeostasis
853 and stress signaling, *Cell metabolism*, 15 (2012) 623-634.

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



B



C

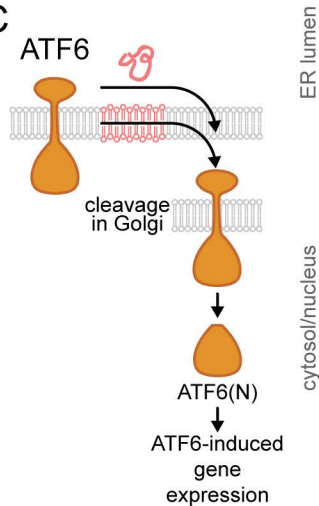


Figure 1

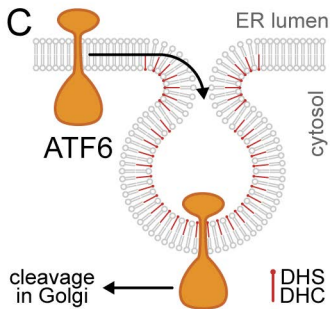
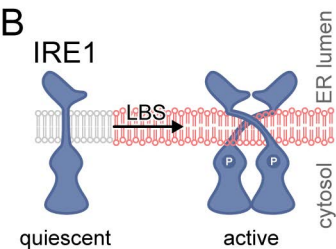
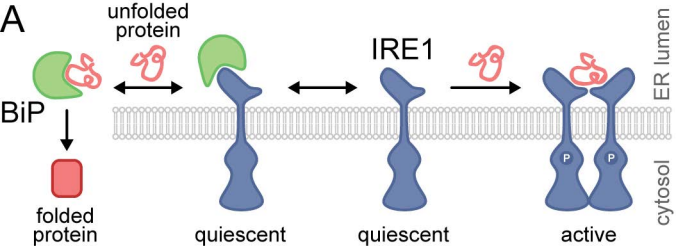


Figure 2

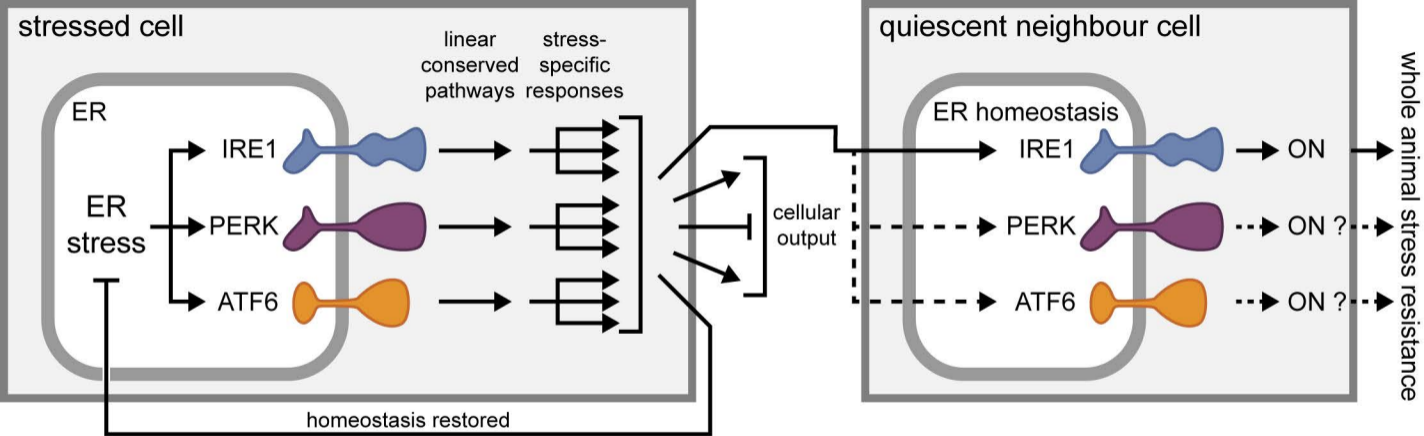


Figure 3