




## REVIEW ARTICLE

# Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications

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## Keywords

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The endoplasmic reticulum (ER) is a membranous intracellular organelle and the first compartment of the secretory pathway. As such, the ER contributes to the production and folding of approximately one-third of cellular proteins, and is thus inextricably linked to the maintenance of cellular homeostasis and the fine balance between health and disease. Specific ER stress signalling pathways, collectively known as the unfolded protein response (UPR), are required for maintaining ER homeostasis. The UPR is triggered when ER protein folding capacity is overwhelmed by cellular

## Abbreviations

4-PBA, 4-phenylbutyric acid; ALS, amyotrophic lateral sclerosis; ATF4, activating transcription factor 4; ATF6f, cytosolic domain of ATF6; ATF6 $\alpha$ , activating transcription factor 6  $\alpha$ ; ATF6 $\beta$ , activating transcription factor 6  $\beta$ ; BBF2H7, cAMP responsive element-binding protein 3 like 2; BiP, binding immunoglobulin protein (gene *GRP78*); bZIP, basic-leucine zipper; CHOP, CAAT/enhancer-binding protein (C/EBP) homologous protein; CRCL, chaperone-rich cell lysate; CREB3L3, cAMP responsive element-binding protein 3 like 3; CREB, cAMP response element-binding protein; eIF2B, eukaryotic translation initiation factor 2B; eIF2 $\alpha$ , eukaryotic translation initiation factor 2 $\alpha$ ; ERAD, ER-associated protein degradation; ER, endoplasmic reticulum; ERN1, endoplasmic reticulum to nucleus signalling 1; ERN2, endoplasmic reticulum to nucleus signalling 2; ERO-1, ER oxidoreductin 1; ER $\alpha$ , oestrogen receptor  $\alpha$ ; GADD34, growth arrest and DNA-damage-inducible 34; GRP78, glucose-regulated protein 78; GSH, glutathione; IBD, inflammatory bowel disease; IRE1 $\alpha$ , inositol-requiring enzyme 1  $\alpha$ ; IRE1 $\beta$ , inositol-requiring enzyme 1  $\beta$ ; LUMAN, cAMP responsive element-binding protein 3 or CREB3; MAM, mitochondria-associated membrane; MBTPS1, membrane bound transcription factor peptidase, site 1; MBTPS2, membrane bound transcription factor peptidase, site 2; MDM1/SNX13, mitochondrial distribution and morphology 1/sorting nexin 13; mTOR, mammalian target of rapamycin; N-ATF6, N-terminal portion of ATF6 or ATF6f; NF-Y, nuclear transcription factor Y; NGLY1, N-glycanase; NPR, NADPH-P450 reductase; OASIS, cAMP responsive element-binding protein 3 like 1; ORAI1, calcium release-activated calcium channel protein 1; PDI, protein disulfide isomerase; p-eIF2 $\alpha$ , phospho-eIF2 $\alpha$ ; PERK, protein kinase RNA-like (PKR-like) endoplasmic reticulum kinase; PKR, protein kinase RNA-activated; PM, plasma membrane; PP1, protein phosphatase type 1; qPCR, quantitative polymerase chain reaction; RER, rough endoplasmic reticulum; RIDD, regulated IRE1-dependent decay; ROS, reactive oxygen species; SEC22b, vesicle-trafficking protein SEC22b; SERCA, sarco/endoplasmic reticulum ATPase Ca<sup>2+</sup>-ATPase; SER, smooth endoplasmic reticulum; TAD, transcriptional activation domain; TRAF2, tumour necrosis factor receptor-associated factor 2; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid; UPR, unfolded protein response; WT, wild-type; XBP1s, spliced isoform of XBP1; XBP1u, unspliced isoform of XBP1; XBP1, X-box binding protein 1.

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demand and the UPR initially aims to restore ER homeostasis and normal cellular functions. However, if this fails, then the UPR triggers cell death. In this review, we provide a UPR signalling-centric view of ER functions, from the ER's discovery to the latest advancements in the understanding of ER and UPR biology. Our review provides a synthesis of intracellular ER signalling revolving around proteostasis and the UPR, its impact on other organelles and cellular behaviour, its multifaceted and dynamic response to stress and its role in physiology, before finally exploring the potential exploitation of this knowledge to tackle unresolved biological questions and address unmet biomedical needs. Thus, we provide an integrated and global view of existing literature on ER signalling pathways and their use for therapeutic purposes.

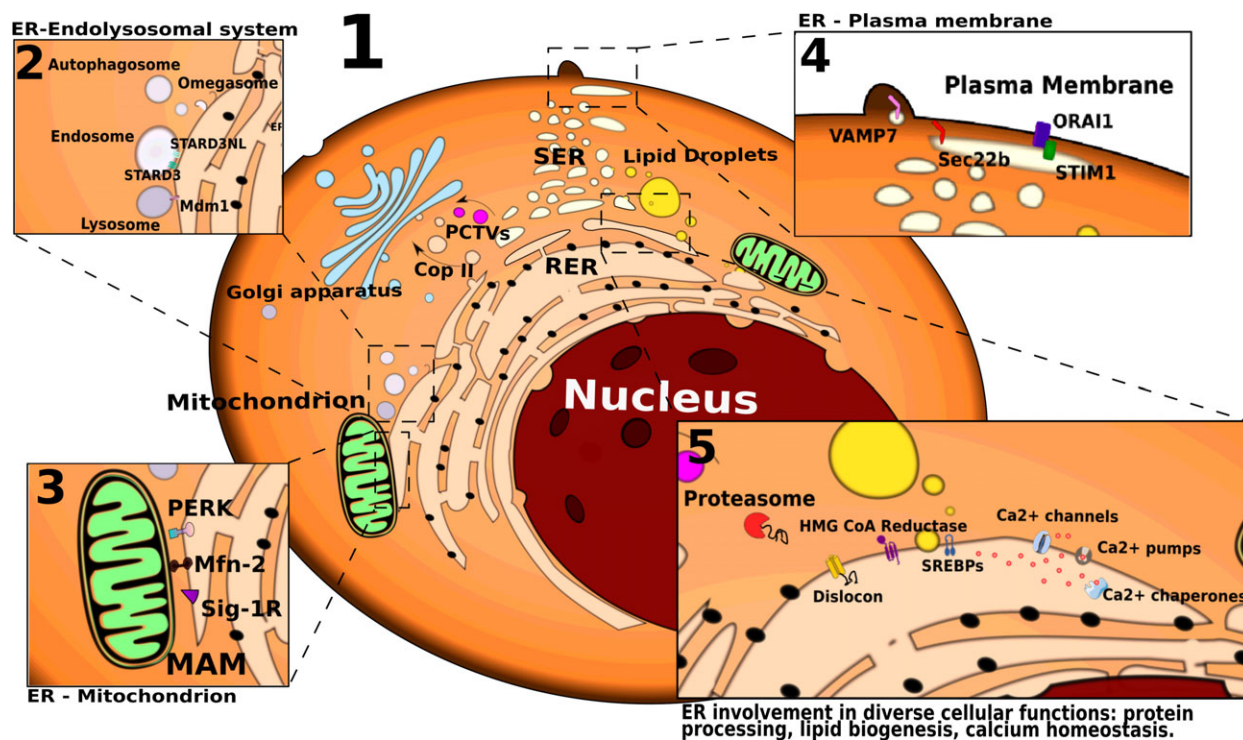
## Introduction

The endoplasmic reticulum (ER) is a cellular organelle that was first visualized in chicken fibroblast-like cells using electron microscopy and was described as a 'delicate lace-work extending throughout the cytoplasm' [1]. Its current name was coined almost 10 years later by Porter in 1954 [2]. The ER appears as a membranous network of elongated tubules and flattened discs that span a great area of the cytoplasm [3]. This membrane encloses the ER lumen and allows for the transfer of molecules to and from the cytoplasm.

## ER structure

The ER is classically divided into the rough ER (RER) and smooth ER (SER), depending on the presence or absence of ribosomes on the cytosolic face of the membrane respectively. The SER and RER can exist either as interconnected or spatially separated compartments [4]. More recently, a novel classification was proposed based on membrane structure rather than appearance. According to this classification, the ER comprises the nuclear envelope, sheet-like cisternae and a polygonal array of tubules connected by three-way junctions [5]. A striking difference between these ER structures is the curvature of the membrane,

whereby ER tubules possess a high membrane curvature compared to the sheets of the nuclear envelope and cisternae. The ER occupies an extensive cell-type-specific footprint within the cell and is in contact with many other intracellular organelles. It forms physical contact sites with mitochondria named mitochondria-associated membranes (MAMs), which play a crucial role in  $\text{Ca}^{2+}$  homeostasis [6]. It also comes in contact with the plasma membrane (PM), an interaction regulated by proteins like stromal interaction molecule 1 in the ER and calcium release-activated calcium channel protein 1 in the PM which are controlled by  $\text{Ca}^{2+}$  levels [7]. Vesicle-trafficking protein SEC22b (SEC22b) and vesicle-associated membrane protein 7 are also involved in the stabilization of ER-PM contacts and PM expansion [8]. The ER also interacts with endosomes [9] and is tethered by StAR-related lipid transfer protein 3 and StAR-related lipid transfer protein 3 [10], which also contribute to cholesterol maintenance in endosomes [11]. Interestingly, an ER interaction with the endolysosomal system, mediated by the mitochondrial distribution and morphology 1/sorting nexin 13 (MDM1/SNX13) complex [12], suggests ER involvement in autophagy. Indeed, a specialized ER structure called the omegasome forms contact sites with the phagophore, which elongates and becomes a mature autophagosome [13,14] (Fig. 1). In this way, the ER on its own or in coordination with other cell



**Fig. 1.** ER molecular machines and contact sites with other organelles. The ER is primarily subdivided into the SER and RER, with the latter characterized by the presence of ribosomes at its cytosolic surface. Alternatively, the ER has been recently classified into the nuclear envelope, ER sheet-like cisternae and tubular ER (panel 1). The ER forms multiple membrane contact sites with other organelles, including the endosomes and lysosomes (through STARD3, STARD3NL, Mdm1; panel 2), the mitochondria (through Mfn-2, Sig-1R, PERK; panel 3), and the PM (through ORAI1, STIM1, Sec22b, VAMP7; panel 4) with various functional implications. The ER plays instrumental roles in secretory and transmembrane protein folding and quality control, protein and lipid trafficking, lipid metabolism, and  $\text{Ca}^{2+}$  homeostasis, all of these processes being mediated by a diverse series of ER resident proteins (schematically depicted in panels 1 and 5).

organelles exerts its multifaceted roles in the functionality of the cell as it is discussed in the next sections.

## ER functions

The ER is involved in many different cellular functions. It acts as a protein synthesis factory, contributes to the storage and regulation of calcium, to the synthesis and storage of lipids, and to glucose metabolism [3]. These diverse functions indicate a pivotal role for the ER as a dynamic ‘nutrient sensing’ organelle that coordinates energetic fluctuations with metabolic reprogramming responses, regulating metabolism and cell fate decisions (Fig. 1).

### Protein folding and quality control

The ER is involved in secretory and transmembrane protein synthesis, folding, maturation, quality control and degradation, and ensures that only properly folded proteins are delivered to their site of action [15]. About 30% of all proteins are cotranslationally targeted to the

ER [16] where they are exposed to an environment abundant in chaperones and foldases that facilitate their folding, assembly and post-translational modification before they are exported from the ER [16]. Protein processing within the ER includes signal sequence cleavage, N-linked glycosylation, formation, isomerization or reduction of disulfide bonds [catalysed by protein disulfide isomerases (PDIs), oxidoreductases], isomerization of proline or lipid conjugation, all of which ultimately result in a properly folded conformation [16–19]. Misfolded proteins are potentially detrimental to cell function and are therefore tightly controlled. Although protein misfolding takes place continually, it can be exacerbated during adverse intrinsic and environmental conditions. The ER has developed quality control systems to ensure that there are additional opportunities to correct misfolded proteins or, if terminally misfolded, to be disposed of by the cell. Terminally misfolded secretory proteins are eliminated by a process called ER-associated degradation (ERAD) [20]. Proteins are first recognized by an ER resident luminal and transmembrane protein machinery, then retrotranslocated into

the cytosol by a channel named dislocon [21] and the cytosolic AAA+ ATPase p97 [22], deglycosylated by *N*-glycanase (NGLY1; [23]) and targeted for degradation via the ubiquitin–proteasome pathway [20,24,25] (Fig. 1).

### Lipid synthesis

The ER also plays essential roles in membrane production, lipid droplet/vesicle formation and fat accumulation for energy storage. Lipid synthesis is localized at membrane interfaces and organelle contact sites, and the lipid droplets/vesicles are exported in a regulated fashion. The ER dynamically changes its membrane structure to adapt to the changing cellular lipid concentrations. The ER contains the sterol regulatory element-binding protein family of cholesterol sensors ensuring cholesterol homeostasis [26]. This compartment also hosts enzymes catalysing the synthesis of cell membrane lipid components, namely sterols, sphingolipids and phospholipids [27]. The synthesis of those lipids from fatty acyl-CoA and diacylglycerols takes place at the ER membrane [28], which also hosts 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, the rate-limiting enzyme of the mevalonate/isoprenoid pathway that produces sterol and isoprenoid precursors [29]. Precursors made by ER membrane-localized enzymes are subsequently converted into structural lipids, sterols, steroid hormones, bile acids, dolichols, prenyl donors and a myriad of isoprenoid species with key functions for cell metabolism. Interestingly, MAMs have been identified as a privileged site of sphingolipid synthesis [30] (Fig. 1).

### ER export

Most of the proteins and lipids synthesized in the ER must be transported to other cellular structures, which occurs mostly through the secretory pathway. To maintain the constant anabolic flux, export needs to be tightly regulated, and defects in secretion can lead to serious structural and functional consequences for the ER. Central to this export process is the generation of ER COPII transport vesicles, named after the family of proteins that shapes and coats them [31]. In addition to COPII vesicle transport, several other mechanisms of lipid export have been described. A variety of lipids can be transported by nonvesicular mechanisms; for example, large lipoprotein cargo has been shown to be exported out of the ER in another type of vesicle termed prechylomicron transport vesicles [32] or to accumulate in lipid droplets (Fig. 1).

### Ca<sup>2+</sup> homeostasis

Ca<sup>2+</sup> is involved as a secondary messenger in many intracellular and extracellular signalling networks, playing an essential role in gene expression, protein synthesis and trafficking, cell proliferation, differentiation, metabolism or apoptosis [33]. ER, as the main cellular compartment for Ca<sup>2+</sup> storage, plays a pivotal role in the regulation of Ca<sup>2+</sup> levels and reciprocally many ER functions are controlled in a Ca<sup>2+</sup>-dependent way, thereby regulating the calcium homeostasis of the whole cell [34]. Consequently, both ER and cytosolic Ca<sup>2+</sup> concentrations need to be highly spatiotemporally regulated in order for the ER to maintain a much increased physiological intraluminal Ca<sup>2+</sup> concentration and oxidizing redox potential than the cytoplasm. To modulate these levels, the ER employs a number of mechanisms that control Ca<sup>2+</sup> concentration on both sides of the membrane: (a) ER membrane ATP-dependent Ca<sup>2+</sup> pumps for cytosol-to-lumen transport; (b) ER luminal Ca<sup>2+</sup>-binding chaperones for sequestering free Ca<sup>2+</sup>; and (c) ER membrane channels for the regulated release of Ca<sup>2+</sup> into the cytosol. These mechanisms are facilitated by a tight communication between the ER and other organelles, such as the PM and the mitochondria, thereby supporting the cell needs.

Traditionally thought as a site of protein synthesis, recent evidence has established the involvement of the ER in many different cellular functions: from novel roles in lipid metabolism to connections with cytoskeletal structures or roles in cytoplasmic streaming, our view of the ER keeps rapidly expanding, placing it increasingly as a key organelle governing the whole cellular metabolism.

### Perturbing ER functions

Conditions that disrupt ER homeostasis create a cellular state commonly referred to as 'ER stress'. The cellular response to ER stress involves the activation of adaptive mechanisms to overcome stress and restore ER homeostasis. This response is dependent on the perturbing agent/condition and the intensity/duration of the stress [35].

### Intrinsic ER perturbations

Cell autonomous mechanisms can lead to ER perturbation and examples of this can be seen in several diseases, including cancer, neurodegenerative diseases and diabetes. The hallmarks of cancer such as genetic instability and mutations [36] can result in constitutive

activation of ER stress response pathways leading to cell growth, proliferation, differentiation and migration. In addition, the uncontrolled, rapid growth of cancer cells requires high protein production rates with a consequent impact on ER systems [37]. Many cancers have a high mutation load which results in an intrinsically higher level of ER stress. For example, melanoma has the highest mutation burden of any cancer and the sheer numbers of mutated proteins are a source of intrinsically higher ER stress levels. In chronic myeloid leukaemia, the fusion protein produced by the Philadelphia chromosome, BCR-ABL1, is a constitutively active oncoprotein that enhances cell proliferation and interferes with Ca<sup>2+</sup>-dependent apoptotic response [38]. In addition, mutation-driven ER stress can also induce senescence that contributes to chemoresistance [39]. ER stress has also been linked to several neurodegenerative diseases. For example, mutations in the ER resident vesicle-associated membrane protein-associated protein B in familial amyotrophic lateral sclerosis (ALS) are linked to induction of motor neuron death mediated by the alteration of ER stress signalling [40,41]. On the other hand, secretory cells such as pancreatic  $\beta$  cells have a highly developed ER to manage insulin production and release in response to increases in blood glucose. The C96Y insulin variant leads to its impaired biogenesis and ER accumulation in the Akita mouse. As the ER cannot cope with the mutation induced stress, beta cells die and type 1 diabetes develops [42,43]. Insulin mutation-related ER stress was also reported in neonatal diabetes [44,45].

## Extrinsic perturbations

### Microenvironmental stress

In tumours, the ER stress observed in rapidly proliferating cells is compounded by the fact that increased proliferation eventually depletes the microenvironment of nutrients and oxygen, causing local microenvironmental stress and resulting in hypoxia, starvation and acidosis, all of which cause ER stress and perturb protein, and possibly lipid synthesis [46]. Nutrient deprivation, and particularly glucose starvation, at least in part, promotes ER stress by impairing glycosylation.

### Exposure to ER stressors

Several small molecules that induce ER stress through a variety of mechanisms have been identified [47,48]. Stressors such as tunicamycin [49,50], or 2-deoxyglucose [51] target the N-linked glycosylation of proteins,

whereas dithiothreitol inhibits protein disulfide bond formation [52]. Alternatively, Brefeldin A impairs ER-to-Golgi trafficking, thus causing a rapid and reversible inhibition of protein secretion [53]. Targeting the Sarco/ER Ca<sup>2+</sup>-ATPase (SERCA) with compounds, such as thapsigargin and cyclopiazonic acid [54,55], induces ER stress by reducing ER Ca<sup>2+</sup> concentration and impairing protein folding capacity.

### Exposure to enhancers of ER homeostasis

Conversely, other molecules have been found that can alleviate ER stress. These include small molecules, peptides and proteostasis regulators. The frequently used 4-phenylbutyric acid (4-PBA) reduces the accumulation of misfolded proteins in the ER [56]. Tauroursodeoxycholic acid (TUDCA) is an endogenous bile acid able to resolve ER stress in islet cells [57]. TUDCA is the taurine conjugate of ursodeoxycholic acid (UDCA), an FDA-approved drug for primary biliary cirrhosis that is also able to alleviate ER stress [58]. The precise mode of action of such proteostasis modulators still remains elusive.

### Temperature

Body temperature is crucial for the viability of metazoans; normal mammalian physiological temperatures are 36–37 °C. Deviations from this range can disrupt cellular homeostasis causing protein denaturation and/or aggregation [59]. Moreover, an acute increase in temperature, known as heat shock, causes the fragmentation of both ER and Golgi [59]. Heat preconditioning at mildly elevated temperatures (up to 40 °C) in mammalian cellular and animal models has been shown to lead to the development of thermotolerance, which is associated with an increase in the expression of several heat shock proteins and ER stress markers [60,61]. In addition, moderate hypothermia (28 °C) induces mild ER stress in human pluripotent stem cells, the activation of which may be sufficient to protect against severe stress through an effect known as ER hormesis [62,63].

### Reactive oxygen species production and other perturbations

Several external agents can induce intracellular reactive oxygen species (ROS) production, and when ROS production exceeds the antioxidant capacity oxidative stress negatively affects protein synthesis and ER homeostasis [64]. ROS, including free radicals, are generated by the UPR-regulated oxidative folding

machinery in the ER [65] and in the mitochondria [66]. In this context, increased mitochondrial respiration and biogenesis promotes survival during ER stress through a reduction of ROS [67]. The ER provides an oxidizing environment to facilitate disulfide bond formation and this process is believed to contribute to as much as 25% of the overall ROS generated [68,69]. The interconnection between the ER and ROS is mediated by signalling pathways which involve glutathione (GSH)/glutathione disulfide, NADPH oxidase 4, NADPH-P450 reductase,  $\text{Ca}^{2+}$ , ER oxidoreductin 1 (ERO1) and PDI [70]. The latter, in particular, has been found upregulated in the central nervous system of Alzheimer's disease patients thus highlighting the relevance of these pathways in neurodegenerative disease [71]. Overall, from the sections above it is apparent that directly or indirectly impaired ER function contributes to disease development and treatment resistance.

## ER stress consequences

In response to ER stress, cells trigger an adaptive signalling pathway called the unfolded protein response (UPR), which acts to help cells to cope with the stress by attenuating protein synthesis, clearing the unfolded/misfolded proteins and increasing the capacity of the ER to fold proteins.

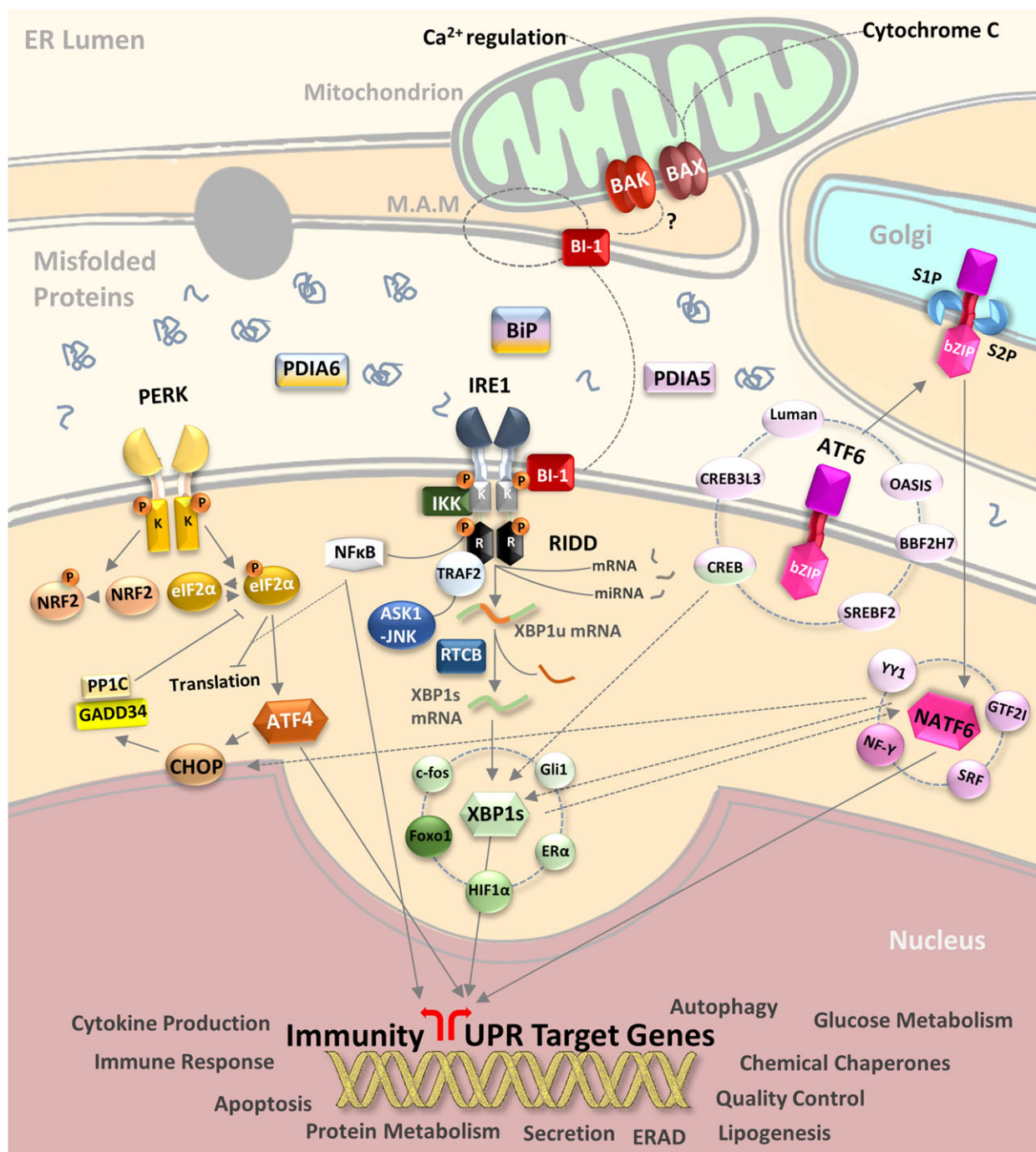
### The UPR

The UPR is a cellular stress response originating in the ER and is predominantly controlled by three major sensors: inositol requiring enzyme 1 (IRE1), protein kinase RNA-activated (PKR)-like ER kinase (PERK) and activating transcription factor 6 (ATF6). The ER luminal domains of all three ER stress sensors are normally bound by the ER resident chaperone, heat shock protein A5 [heat shock protein family A (Hsp70) member 5, also known as glucose-regulated protein 78 (GRP78) and binding immunoglobulin protein (gene *GRP78*) (BiP)], keeping them in an inactive state [72,73]. Accumulating misfolded proteins in the ER lumen engage BiP thus releasing the three sensors. A FRET UPR induction assay, developed to quantify the association and dissociation of the IRE1 luminal domain from BiP upon ER stress [74], demonstrated that the ER luminal co-chaperone ERdj4/DNAJB9 represses IRE1 by promoting a complex between BiP and the luminal stress-sensing domain of IRE1 $\alpha$  [75]. Moreover, it has recently been reported that another ER luminal chaperone, Hsp47, displaces BiP from the IRE1 UPRosome to promote its oligomerization [76]. Once released from

BiP, IRE1 and PERK homodimerize or oligomerize and trans-autophosphorylate to activate their downstream pathways [72]. In contrast, BiP dissociation from AFT6 reveals an ER export motif [73] which facilitates its translocation to the Golgi apparatus [77]. This 'competition model' of UPR activation assumes that BiP acts as a negative regulator of UPR signalling. However, other BiP-dependent or independent models have been proposed (reviewed in [78]; Fig. 2).

### IRE1 signalling

In humans, there are two paralogues of IRE1 (IRE1 $\alpha$  and  $\beta$ ), encoded by endoplasmic reticulum to nucleus signalling 1 and 2 (*ERN1* and *ERN2*), respectively [79–81]. Both human IRE1 isoforms share significant sequence homology (39%) [20]. IRE1 $\alpha$  (referred to IRE1 hereafter) is ubiquitously expressed; however, inositol-requiring enzyme 1  $\beta$  (IRE1 $\beta$ ) expression is restricted mainly to the gastrointestinal tract and the pulmonary mucosal epithelium [82,83]. *Ern1* knockout (KO) in mice is embryonic lethal due to growth retardation and defects in liver organogenesis and placental development [84] while *Ern2* KO mice develop colitis of increased severity and shorter latency [82] but are otherwise histologically indistinguishable from the *Ern2*WT mice. BiP dissociation, caused by accumulating unfolded proteins, triggers IRE1 oligomerization and activation of its cytosolic kinase domain. The oligomers position in close proximity, in a face-to-face orientation, enabling trans-autophosphorylation. This face-to-face configuration is adopted by both human and murine IRE1 [85,86]. Phosphorylation in the activation loop of the kinase domain, specifically at Ser724, Ser726 and Ser729, is not only necessary to activate its cytosolic RNase domain [87] but is also required to initiate recruitment of tumour necrosis factor receptor-associated factor 2 (TRAF2) and JNK pathway signalling [88]. The IRE1 cytosolic domain, which is highly homologous with RNase L [89], induces a selective cleavage of dual stem loops within the X-box binding protein 1 (XBP1) mRNA [79,90,91]. Therefore, IRE1, in a spliceosome independent-manner, but together with the tRNA ligase RNA 2',3'-cyclic phosphate and 5'-OH ligase [92–97], catalyses the splicing of a 26 nucleotide intron from human XBP1 mRNA to produce spliced isoform of XBP1 (XBP1s) [90,91]. XBP1s is a basic leucine zipper (bZIP) transcription factor [98–100] and the unspliced isoform of XBP1 (XBP1u) is unable to activate gene expression due to lack of a transactivation domain [91]. The N-terminal region of XBP1u contains a basic region and a leucine zipper domain



**Fig. 2.** Signalling the UPR and downstream pathways. The 3ER stress sensors (PERK, IRE1, ATF6) upon release from BiP, PDIA5, 6 initiate signalling cascades through transcription factor production (ATF4, XBP1s, ATF6f) and associated processes such as RIDD, NFκB activation and ERAD to address the misfolded protein load on the ER. By modulating transcriptional output and translational demand the UPR attempts to re-establish ER protein folding homeostasis and promote cell survival. If ER stress cannot be resolved then mechanisms are triggered to promote cell death.

involved in dimerization and DNA binding [91,98,100,101]. The XBP1u C-terminal region contains a P (proline), E (glutamic acid), S (serine) and

T (threonine) motif which destabilizes proteins (ubiquitin-dependent proteolysis) and contributes to its short half-life [98,101–103]. The N-terminal region

also contains two other domains: a hydrophobic region that targets XBP1u to the ER membrane and a domain that promotes efficient XBP1 splicing [104–106] and cleavage [103] by pausing XBP1 translation. IRE1-mediated splicing of XBP1 mRNA results in an open reading frame-shift inducing the expression of a transcriptionally active and BP1s [90,91,101]. XBP1u has been reported to negatively regulate XBP1s transcriptional activity as well as to promote the recruitment of its own mRNA to the ER membrane through the partial translation of its N-terminal region [107,108]. XBP1s directs the transcription of a wide range of targets including the expression of chaperones, foldases and components of the ERAD pathway, in order to relieve ER stress and restore homeostasis [109,110]. However, XBP1s can also participate in the regulation of numerous metabolic pathways such as lipid biosynthesis [111–113], glucose metabolism [114–118], insulin signalling [117,119,120], redox metabolism [121], DNA repair [122] and it influences cell fate including cell survival [123], cell differentiation [124–128] and development [126,129–131]. Although there is strong evidence pointing to a key role for XBP1 in multiple cellular functions, the exact mechanisms by which XBP1 mediates gene transactivation are still elusive. Indeed, in addition to the known interaction of the XBP1s transactivation domain with RNA polymerase II, other mechanisms could exist. For example, XBP1 can physically interact with many other transcription factors such as AP-1 transcription factor subunit [132], oestrogen receptor  $\alpha$  (ER $\alpha$ ) [133], GLI-family zinc finger 1 [134], SSX family member 4 [134], forkhead box O1 [114], ATF6 [135], cAMP response element-binding protein (CREB)/ATF [135] and hypoxia inducible factor 1 alpha subunit [136] (Fig. 2).

The RNase activity of IRE1 can also efficiently target other transcripts through a mechanism called regulated IRE1-dependent decay (RIDD) [137]. Analysis of the *in vitro* RNase activity of wild-type (WT) vs mutant IRE1 led to the discovery of a broad range of other IRE1 substrates [138,139] and, interestingly, it was noted that IRE1 can also degrade its own mRNA [140]. RIDD is a conserved mechanism in eukaryotes [137,141–145] by which IRE1 cleaves transcripts containing the consensus sequence (CUGCAG) accompanied by a stem-loop structure [142,146]. The cleaved RNA fragments are subsequently rapidly degraded by cellular exoribonucleases [141,147]. RIDD is required for the maintenance of ER homeostasis by reducing ER client protein load through mRNA degradation [137,141,142]. Recently, it has been proposed that there is basal activity of RIDD [138] which increases

progressively with the severity of ER stress. However, this hypothesis needs further experimental validation. Interestingly, IRE1 $\beta$  was found to selectively induce translational repression through the 28S ribosomal RNA cleavage [81] demonstrating that IRE1 $\alpha$  and IRE1 $\beta$  display differential activities [148]. Characterizing RIDD activity, particularly *in vivo*, has proven difficult due to the complex challenge of separating the RIDD activity from the XBP1 splicing activity of IRE1. In addition, basal RIDD can only target specific mRNA substrates, as full activation and subsequent targeting of further transcripts requires strong ER stress stimuli (Fig. 2).

### PERK signalling

PERK was identified in rat pancreatic islets as a serine/threonine kinase and, similar to PKR, heme regulated initiation factor 2 alpha kinase and general control nonderepressible 2, can phosphorylate eIF2 $\alpha$  [149,150]. PERK is ubiquitously expressed in the body [149] and has an ER luminal domain as well as a cytoplasmic kinase domain [150]. BiP detachment from the ER luminal domain leads to oligomerization [72], trans-autophosphorylation and activation of PERK [151]. Active PERK phosphorylates eIF2 $\alpha$  on serine 51 [150]. eIF2 $\alpha$  is a subunit of the eIF2 heterotrimer [152,153] which regulates the first step of protein synthesis initiation by promoting the binding of the initiator tRNA to 40S ribosomal subunits [154]. However, eIF2 $\alpha$  phosphorylation by PERK inhibits eukaryotic translation initiation factor 2B (eIF2B) activity and thereby downregulates protein synthesis [155]. Blocking translation during ER stress consequently reduces the protein load on the ER folding machinery [156].

Remarkably, some transcripts are translated more efficiently during PERK-dependent global repression of translation initiation. The ubiquitously expressed activating transcription factor 4 (ATF4) [157], whose transcript contains short upstream open reading frames (uORFs) [158], is normally inefficiently translated from the protein-coding AUG [159]. However, attenuation of translation from uORFs shifts translation initiation towards the protein coding AUG, resulting in more efficient synthesis of ATF4 [158]. ATF4 can then bind to the C/EBP-ATF site in the promoter of CAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP)/GADD153 [160] and induce its expression [158]. ATF4 and CHOP directly induce genes involved in protein synthesis and the UPR [161], but conditions under which ATF4 and CHOP increase protein synthesis can result in ATP depletion, oxidative stress and cell death [162]. eIF2 $\alpha$



phosphorylation (p-eIF2 $\alpha$ ) can also directly enhance the translation of CHOP [163,164] and other proteins involved in the ER stress response, as reviewed in [165]. For example, growth arrest and DNA-damage-inducible 34 (GADD34) [166,167] is positively regulated by eIF2 $\alpha$  phosphorylation [168] and likewise transcriptionally induced by ATF4 [169] and CHOP [170]. Interestingly, GADD34 interacts with the catalytic subunit of type 1 protein serine/threonine phosphatase (PP1) [171], which dephosphorylates eIF2 $\alpha$  thereby creating a negative feedback loop that antagonizes p-eIF2 $\alpha$ -dependent translation inhibition and restores protein synthesis [169,170,172]. The translational arrest induced by p-eIF2 $\alpha$  reduces protein load in ER lumen and conserves nutrients, while ATF4 driven expression of adaptive genes involved in amino acid transport and metabolism, protection from oxidative stress, protein homeostasis and autophagy together help the cell to cope with ER stress [173,174]. However, sustained stress changes the adaptive response to a prodeath response and ultimately, the phosphorylation status of eIF2 $\alpha$  appears to codetermine the balance between prosurvival or prodeath signalling [175,176]. This is accomplished by the above mentioned delayed feedback through which the interplay of GADD34, ATF4 and CHOP results in the activation of genes involved in cell death, cell-cycle arrest and senescence [177–180] (Fig. 2).

### ATF6 signalling

The transcription factor ATF6, which belongs to an extensive family of leucine zipper proteins [8], is encoded in humans by two different genes: *ATF6A* for ATF6 $\alpha$  [181] and *ATF6B* for ATF6 $\beta$  [153]. After its activation in the ER and export to the Golgi, it is cleaved by the two Golgi-resident proteases membrane bound transcription factor peptidase, site 1 (MBTPS1) and MBTPS1, releasing a fragment of ~ 400 amino acids corresponding to ATF6 cytosolic N-terminal portion (ATF6f). ATF6f comprises a transcriptional activation domain (TAD), a bZIP domain, a DNA-binding domain and nuclear localization signals. In the nucleus, ATF6f induces UPR gene expression [73,182]. Although the two ATF6 paralogs share high homology [153], ATF6 $\beta$  is a very poor activator of UPR genes due to the absence of eight important amino acids in the TAD domain [157]. Indeed, it rather seems to function as an inhibitor by forming heterodimers with ATF6 $\alpha$  [10,158]. Interestingly, ATF6 can modulate gene expression by interacting with other bZIPs, such as CREB [159], cAMP responsive element-binding protein 3 like 3 (CREB3L3) [160], sterol regulatory element-binding

transcription factor 2 [161] and XBP1 [71], and various other transcription factors such as serum response factor [181], components of the nuclear transcription factor Y (NF-Y) complex [159,162,163], yin yang 1 [163,164] and general transcription factor I [165]. Converging with IRE1 and PERK signalling cascades, ATF6 can also induce the expression of XBP1 and CHOP to enhance UPR signalling [30,166,167]. However, ATF6 is not the only ER-resident bZIP transcription factor. At least five other tissue-specific bZIPs, named Luman, cAMP responsive element-binding protein 3 like 1 (OASIS), cAMP responsive element-binding protein 3 like 2 (BBF2H7), CREB3L3 and CREB, reviewed in [183], are involved in ER stress signalling (Fig. 2), highlighting the regulatory complexity this branch of the ER stress response is subjected to at the organismal level.

### Noncoding RNAs

Noncoding RNAs are connected to the three UPR sensors with effects on both physiological and pathological conditions [184]. These RNA species mostly include microRNAs (miRNAs) and also long noncoding RNAs (lncRNAs). This additional level of regulation works in fact in a bidirectional manner. This means that either the UPR sensors themselves or their downstream components can also modulate their expression levels. A certain number of miRNAs have been so far recognized to regulate IRE1, which in turn regulates miRNAs through XBP1s at a transcriptional level and through RIDD activity via degradation. One miRNA regulates PERK expression, while this in turn regulates miRNAs through its downstream targets. ATF6 is also modulated by miRNAs, but only one miRNA has been found under its direct effect. Upstream of IRE1, PERK and ATF6, the BiP chaperone is also regulated by miRNAs but does not control any. In addition to miRNAs, lncRNAs exhibit a similar role regarding the regulation of UPR factors and vice versa. Their levels change in accordance to the cell stress status and depending on the pathophysiological context lead to distinct cell fates. This interconnection between noncoding RNAs and the UPR may contribute to a more complex network but at the same time reveals the existence of fine-tuning mechanisms governing ER stress responses and their effects in cell homeostasis (described in [184]).

### Proximal impact of UPR activation

#### Transcriptional programmes

Each branch of the UPR pathway culminates in transcriptional regulation and, together the UPR's major

transcription factors, ATF6f, XBP1s and ATF4, stimulate many adaptive responses to restore ER function and maintain cell survival [35]. They regulate genes encoding ER chaperones, ERAD factors, amino acid transport and metabolism proteins, phospholipid biosynthesis enzymes, and numerous others [185]. In particular, the IRE1–XBP1 pathway is involved in the induction of ER chaperones and capacity control of ERAD [186] as well as promoting cytoprotection [187] and cleaving miRNAs that regulate the cell death-inducing caspases [188]. ATF6f translocates to the nucleus where it activates genes involved in protein folding, processing, and degradation [185]. ATF4, activated downstream of PERK and p-eIF2 $\alpha$ , increases the transcription of many genes that promote survival under ER stress. Some of these prosurvival genes include genes that are involved in redox balance, amino acid metabolism, protein folding and autophagy [189].

### Translational programmes

Translation is directly impacted by UPR activation under ER stress conditions, particularly by PERK as described above. It also affects the expression of several miRNAs, which may further contribute to translation attenuation or protein synthesis [35]. It has been shown that ER stress can regulate the execution phase of apoptosis by causing the transient induction of inhibitor of apoptosis proteins (IAPs). Several papers have reported that cIAP1, cIAP2 and XIAP are induced by ER stress, and that this induction is important for cell survival, as it delays the onset of caspase activation and apoptosis. PERK induction of cIAPs and the transient activity of PI3K–AKT signalling suggest that PERK not only allows adaptation to ER stress, but it also actively inhibits the ER stress-induced apoptotic programme [190].

### Protein degradation

There are two main protein degradation pathways activated by components of the UPR following ER stress: ubiquitin–proteasome-mediated degradation via ERAD and lysosome-mediated protein degradation via autophagy. ERAD is responsible for removing misfolded proteins from the ER and several genes involved in ERAD are upregulated by ATF6f and XBP1s [185]. ERAD involves the retrotranslocation of misfolded proteins from the ER into the cytosol where they are degraded by the proteasome (see above) [187]. When accumulation of misfolded proteins overwhelms ERAD, autophagy is induced as a secondary response

to limit protein build-up [187,191]. Autophagy is a pathway involved in the degradation of bulk components such as cellular macromolecules and organelles. It involves target recognition and selectivity, sequestering targets within autophagosomes, followed by the fusion of the autophagosome with the lysosome, where targets are then degraded by lysosomal hydrolases [187,192]. The direct link between ER stress and autophagy has been established in both *Saccharomyces cerevisiae* and mammalian cells, where autophagy plays a solely cytoprotective role. The PERK (eIF2 $\alpha$ ) and IRE1 (TRAF2/JNK) branches of the UPR have been implicated in ER stress-induced autophagy in mammalian systems to avoid accumulation of lethal disease-associated protein variants [192]. IRE1–JNK signalling activates Beclin 1, a key player and regulator of autophagy, via the phosphorylation of Bcl-2 and the subsequent dissociation from Beclin 1. This then leads to the activation of ATG proteins required for the formation of the autophagolysosome [193]. Overall, these mechanisms decrease the build-up of improperly folded proteins in the ER thus allowing adaptive and repair mechanisms to re-establish homeostasis. As the amounts of improperly folded proteins decrease, the UPR switches off. However, the molecular details of UPR attenuation still remain to be further elucidated.

Overall, the three mechanisms describe above decrease the build-up of proteins in the ER which allows adaptive and repair mechanisms to re-establish homeostasis. As the amounts of improperly folded proteins decrease, the UPR switches off. However, the molecular details of UPR attenuation remain to be further elucidated.

### Regulation of MAMs

Mitochondria-associated membranes (MAMs), which are mainly responsible for Ca<sup>2+</sup> homeostasis maintenance as well as lipid transport, mediate the interaction between the ER and mitochondria thereby controlling mitochondrial metabolism and apoptosis [194]. MAMs contain many proteins and transporters which mediate mitochondrial clustering and fusion, such as the dynamin-like GTPase mitofusin-2 (MFN2) [195]. MFN2 interacts with PERK, serving as an upstream modulator and thereby regulating mitochondrial morphology and function as well as the induction of apoptosis [196]. Furthermore, the cytosolic domain of PERK serves as an ER-mitochondria tether, thus facilitating ROS-induced cell death [197]. The sigma 1 receptor (Sig-1R) is located in the MAMs and forms a complex with BiP. Recent studies show that S1R stabilizes IRE1

at the MAMs upon ER stress, promoting its dimerization and conformational change, and prolonging the activation of the IRE1–XBP1 signalling pathway through its long-lasting endoribonuclease activity. Furthermore, mitochondria-derived ROS stimulates IRE1 activation at MAMs [198]. Another MAM component is Bax-inhibitor-1 (BI-1), regulating mitochondrial  $\text{Ca}^{2+}$  uptake and apoptosis. BI-1 is a negative regulator of IRE1–XBP1 signalling and in BI-1 deficient cells there is IRE1 hyperactivation and increased levels of its downstream targets [199]. Apoptosis activation by the UPR results in mitochondrial membrane permeabilization, with the resulting  $\text{Ca}^{2+}$  transfer potentially triggering mitochondrial cytochrome c release [200]. Less well understood are the interactions of the mitochondria with the ER during sublethal ER stress. The latter results in more ER-mitochondria contacts than lethal levels of ER stress, allowing for transfer of  $\text{Ca}^{2+}$  and enhancement of ATP production through increased mitochondrial metabolism [201] (Fig. 1). These evidences demonstrate the importance of the ER-mitochondria communication in regulating the ER homeostasis and in coordinating the cellular response to ER stress, thereby restoring cellular homeostatic condition or leading towards cell death.

### Redox homeostasis

Oxidative stress can be induced through several mechanisms and is critically controlled by the UPR. PERK activity helps to maintain redox homeostasis through phosphorylation of NRF2 which functions as a transcription factor for the antioxidant response [202]. ATF4 also regulates redox control and has been shown to protect fibroblasts and hepatocytes from oxidative stress [173], as well as ensuring that there is an adequate supply of amino acids for protein and GSH biosynthesis [203]. However, in neurons and HEK293 cells ATF4 was shown to induce cell death in response to oxidative stress while CHOP was reported to induce ERO1- $\alpha$ , resulting in ER  $\text{Ca}^{2+}$  release and apoptosis in macrophages [204]. Direct interactions of PDIs with ER stress sensors, protein S-nitrosylation and ER  $\text{Ca}^{2+}$  efflux that is promoted by ROS contribute to redox homeostasis and by extension to the balance between prosurvival and prodeath UPR signalling [205]. As such, these signalling loops are paramount to normal cellular function.

### Global metabolic impact of the UPR

It was recently shown that the UPR and mitochondrial proteotoxic stress signalling pathways converge

on ATF4 to induce the expression of cytoprotective genes [174]. Another pathway regulating energy metabolism is the nutrient-sensing mammalian target of rapamycin (mTOR) signalling hub. mTOR is associated with the UPR through crosstalk with regulatory pathways (reviewed in [206]), and mTOR inhibitors such as rapamycin lead to the activation of PERK signalling, thus favouring cell viability [207]. PERK can also regulate the PI3K–AKT–mTORC1 axis through the activation of AKT. Furthermore, it was observed that mTORC2 plays a role in the inhibition of PERK through AKT activation [208]. Altogether these data suggest that crosstalk between mTOR and the UPR is complex and occurs through multiple pathways.

### Lipid metabolism

The UPR can also be activated by deregulated lipid metabolism. In this regard, the UPR has been shown to be activated in cholesterol-loaded macrophages resulting in increased CHOP signalling and apoptosis [209]. Notably, chronic ER stress leads to insulin resistance and diabetes in obesity. This is caused by alterations in lipid composition which lead to inhibition of SERCA activity and hence ER stress [210]. On the other hand, the UPR is involved in systemic metabolic regulation. Disturbance of ER homeostasis in the liver is involved in hepatic inflammation, steatosis and nonalcoholic fatty liver disease [211]. The PERK–eIF2 $\alpha$  pathway has been reported to regulate lipogenesis and hepatic steatosis. Compromising eIF2 $\alpha$  phosphorylation in mice by overexpression of GADD34 results in reduced hepatosteatosis upon high-fat diet [212]. ATF4 the downstream effector of PERK–eIF2 $\alpha$  pathway has also been suggested to regulate lipid metabolism in hepatocytes in response to nutritional stimuli by regulating expression of genes involved in fatty acid and lipid production [213,214]. Furthermore, it has been demonstrated that the IRE1–XBP1–PDI axis links ER homeostasis with VLDL production which plays an important role in dyslipidaemia [215]. In addition, XBP1 is required for the normal hepatic fatty acid synthesis and it was shown that selective XBP1 deletion in mice resulted in marked hypocholesterolaemia and hypotriglyceridaemia [216]. These studies suggest that ER stress and the UPR are involved in lipid metabolism. Relieving ER stress ameliorates the disease state associated with lipid metabolism alterations, suggesting that targeting ER stress might serve as a therapeutic strategy for treating diseases associated with lipid accumulation.

## Glucose metabolism

It has been suggested that in the liver the PERK–eIF2 $\alpha$  pathway is responsible for disruption of insulin signalling caused by intermittent hypoxia, though IRE1–JNK pathways may still play a role [217]. Adiponectin is widely regarded as a marker of functional glucose metabolism and as a suppressor of metabolic dysfunctions. In hypoxic and ER-stressed adipocytes, reduced adiponectin mRNA levels are observed due to negative regulation by CHOP [218,219]. In  $\beta$ -cells, it was shown that IRE1 is involved in insulin biosynthesis after transient high glucose levels. However, chronic exposure to high glucose leads to full UPR induction and insulin downregulation [220]. IRE1 signalling was shown to be involved in insulin resistance and obesity through JNK activation. In hepatocytes, IRE1-dependent JNK activation leads (a) to insulin receptor substrate 1 (IRS1) tyrosine phosphorylation (pY896) decrease and (b) to AKT activation leading to an increase of IRS1 phosphorylation (pS307), consequently blocking insulin signalling. A role for XBP1 in the pancreas was demonstrated by the fact that  $\beta$ -cell-specific XBP1 mutant mice show hyperglycaemia and glucose intolerance due to decreased insulin release of  $\beta$ -cells [221]. ER stress-induced activation of ATF6 in rat pancreatic beta cells exposed to high glucose, impairs insulin gene expression and glucose-stimulated insulin secretion. Interestingly, knocking down expression of orphan nuclear receptor short heterodimer partner (SHP) previously reported to be involved in beta cell dysfunction by downregulating expression of PDX-1 and RIPE3b1/MafA partly mitigated this effect. However, it remains unclear how ATF6 induces expression of SHP and whether ATF6 alone can directly regulate the expression of insulin, PDX-1 and RIPE3b1/MafA [222]. It has been suggested that physiological impact of ER stress with respect to glucose metabolism depends upon the availability of glucose. Indeed acute glucose availability in beta cells leads to concerted efforts of each branch of UPR to supply insulin, while chronic glucose stimulation leads to depletion of insulin production and beta cell mass due to apoptosis. Moreover, chronic fasting conditions in mice have shown that XBP1s directly activates the promoter of the master regulator of starvation response, PPAR $\alpha$  demonstrating a further link between the UPR and glucose and lipid metabolism [223]. Acquiring further knowledge on link between UPR and metabolic sensor mechanisms will significantly expand the possibility of gaining beneficial metabolic output. Taken together this indicates that the UPR arms are critical

for the cell to regulate metabolism through regulating mTOR signalling, lipid homeostasis as well as insulin signalling.

## Downstream impact of UPR activation

The activation of UPR leads to the modulation of many cellular pathways, thereby influencing pro-survival mechanisms as well as processes such as proliferation, differentiation, metabolism and cell death.

## UPR-associated cell death

Following prolonged activation of the UPR, the cellular response switches from pro-survival to pro-death. Several types of cell death, including apoptosis, necrosis/necroptosis and autophagic cell death, can be induced following ER stress.

## Apoptosis

Unresolved ER stress can lead to the activation of either the intrinsic (mitochondrial) or extrinsic [death receptor (DR)] pathways of apoptosis. Both pathways trigger activation of caspase proteases that dismantle the cell, and all of the three branches of the UPR are involved in apoptosis. In the extrinsic pathway, the activation of DRs on the PM leads to the recruitment of caspases to the DRs and their proximity-induced trans-autoactivation. Intrinsic apoptosis involves the release of cytochrome *c* (along with other proapoptotic factors) from the mitochondria, which promotes the formation of a cytosolic protein complex to activate a caspase cascade. This release is controlled by pro- and antiapoptotic members of the BCL-2 protein family. In particular, the BH3-only members of the family including PUMA, NOXA and BIM are pivotal components of ER stress-induced apoptosis [224], and cells deficient in BH3-only proteins are protected against ER stress-induced cell death [190]. ER stress leads to transcriptional upregulation of these proapoptotic molecules resulting in cytochrome *c* release. Both the IRE1 and PERK arms of the UPR have been linked to induction of apoptosis during ER stress. In particular, CHOP, a transcription factor that is downstream of PERK, and a direct target of ATF4, has been implicated in the regulation of apoptosis during ER stress. As discussed in section PERK signalling CHOP-induced expression of GADD34 promotes dephosphorylation of p-eIF2 $\alpha$  reversing translational inhibition and allowing transcription of genes including apoptosis-related genes [172]. CHOP activates

transcription of BIM and PUMA, while it represses transcription of certain antiapoptotic BCL-2 family members such as MCL-1 [225]. In addition, the ATF4/CHOP pathway can increase the expression of other proapoptotic genes, such as TRAIL-R1/DR4 and TRAIL-R2/DR5 which promote extrinsic apoptosis [180]. Apart from CHOP, p53 is also involved in the direct transcriptional upregulation of BH3- only proteins during ER stress. However, the link between p53 activation and ER stress is unclear [226].

Although IRE1–XBP1s signalling is mainly pro-survival, IRE1 can promote apoptosis. Activated IRE1 can interact directly with TRAF2, leading to the activation of apoptosis signal-regulating kinase 1 (ASK1) and its downstream targets c-Jun NH2-terminal kinase (JNK) and p38 MAPK [227,228]. Phosphorylation by JNK has been reported to regulate several BCL-2 family members, including the activation of proapoptotic BID and BIM, and inhibition of antiapoptotic BCL-2, BCL-XL and MCL-1 [229,230]. In addition, p38 MAPK phosphorylates and activates CHOP, which increases expression of BIM and DR5, thereby promoting apoptosis [231,232]. In fact, cell death induction in HeLa cells overexpressing CHOP is dependent on its phosphorylation by p38 MAPK [233]. Interestingly, it was proposed that ER stress and MAPK signalling act in a positive feed-forward relationship, as ER stress induces MAPK signalling which in turn increases ER stress [234]. IRE1 signalling may also contribute to apoptosis induction through prolonged RIDD activity which degrades the mRNA of protein folding mediators [142].

Interestingly, recent studies indicate a role for miRNAs in the induction of apoptosis following prolonged ER stress. For example, miRNA29a which is induced during ER stress via ATF4 results in the downregulation of antiapoptotic Bcl-2 family protein Mcl-1, and thus promotes apoptosis [235]. miRNA7 has also been linked with ER stress-induced apoptosis, where IRE1 reduces miRNA7 levels which results in the stability of a membrane-spanning RING finger protein, RNF183. RNF183 has an E3 ligase domain that then causes the ubiquitination and subsequent degradation of the antiapoptotic member of the BCL-2 family BCL-XL. Following prolonged ER stress, increased expression of RNF183 via IRE1 leads to increased apoptosis [236].

In the last decade, it also became clear that ER stress can profoundly modify the immunological consequences of apoptotic cell death. Accumulating *in vitro* and *in vivo* evidence have highlighted that the activation of the PERK arm of ER stress evoked in response to selected anticancer therapies (including anthracyclines, oxaliplatin, radiation and

photodynamic therapy (reviewed in [237]), drives a danger signalling module resulting in the surface exposure of the ER luminal chaperone calreticulin and the exodus of other danger-associated molecular patterns, eliciting immunogenic cell death (reviewed in [238]).

### Necroptosis

Necroptosis, a programmed form of cell death, is dependent on the activation of receptor-interacting protein kinase 1 (RIPK1), RIPK3 and mixed lineage kinase domain-like (MLKL) protein and has been linked to ER stress. In an *in vivo* mouse model of spinal cord injury, there is induction of necroptosis and ER stress, with localization of MLKL and RIPK3 on the ER in necroptotic microglia/macrophages suggesting a link between necroptosis and ER stress in these cells [239]. Necroptosis is frequently activated downstream of TNFR1 when apoptosis is blocked [240]. This has been linked to ER stress-induced necroptosis whereby tunicamycin kills L929 murine fibrosarcoma cells by caspase-independent, death ligand-independent, TNFR1-mediated necroptosis [241].

### Autophagic cell death

Endoplasmic reticulum stress has also been connected to autophagic cell death. Autophagy not only promotes cell survival, but can also mediate nonapoptotic cell death under experimental conditions when apoptosis is blocked, or in response to treatments that specifically trigger caspase-independent autophagic cell death [192]. IRE1 $\alpha$  mediated TRAF2 and ASK1 recruitment, and subsequent JNK activation mediates autophagy. JNK-mediated phosphorylation of BCL-2 releases Beclin-1 (while XBP1s also transcriptionally upregulates its expression), which interacts with the ULK1 complex to promote vesicle nucleation that leads to the formation of the autophagosome [242]. Activated PERK can induce autophagy through ATF4 by inducing vesicle elongation while Ca<sup>2+</sup> release from the ER lumen through the IP3R can relieve mTOR inhibition on the ULK1 complex [187].

### UPR-associated morphological changes

Endoplasmic reticulum stress causes morphological changes in cellular models. Experiments to date have largely focused on the morphologies associated with apoptotic and autophagic cell death resulting from UPR activation. UPR-regulated flattening and rounding of cells, indicative of cell death, has been observed in many model systems, with traditional caspase-

dependent apoptosis being responsible [200,243–248]. These morphological changes can be reversed by physiological and pharmacological ER stress relief [247,249]. Both IRE1 and PERK arms of the UPR have been implicated in the observed changes [193,243,244,247,249–251]. As described above, programmed cell death and its associated morphological changes have become a focal and much researched outcome of the use of UPR-inducing cytotoxic agents.

An intensively studied consequence of ER stress is the epithelial to mesenchymal transition (EMT) and its role in cancer invasion and metastasis. EMT is an essential component of tissue repair following wounding, allowing for the migration of new healthy cells into any lesions that have occurred. Morphological changes indicative of EMT have been observed in multiple cell models under physiologically relevant stress (e.g. hypoxia) and pharmacological induction of ER stress [252–255]. The IRE1–XBP1 pathway has been reported to negatively regulate the traditional epithelial marker E-cadherin, while positively regulating the mesenchymal marker *N*-cadherin in models of colorectal, breast and pulmonary fibrosis [254,256,257]. Breast cancer and pulmonary fibrosis models showed an IRE1–XBP1-dependent regulation of mesenchymal promoting transcription factor SNAIL that is responsible for EMT [254,256]. Human mammary epithelial cells undergo EMT in response to PERK activation, and PERK-mediated phosphorylation of eIF2 $\alpha$  is required for invasion and metastasis [258]. Other ER stress-regulated pathways have been proposed to act in the EMT in cellular models, including autophagy and activation of c-SRC kinase in tubular epithelial cells [259] and the compensatory activation of the NRF-2/HO-1 antioxidative stress response pathway in HT-29 and DLD-1 colon cancer cells [252]. Therefore, UPR signalling pathways appear to induce morphological changes indicative of EMT. These data have generated interest in the field of cancer research where the pharmacological inhibition of UPR components might be used to reduce tumour invasiveness and metastasis.

### Hormone production

The tissues and cells of the endocrine system responsible for hormone production and extracellular signalling often have a high protein load, resulting in ER stress and activation of the UPR. OASIS (CREB3L1) and ATF6 $\alpha$  have been shown to regulate arginine vasopressin (AVP), a potent vasoconstrictor, in murine and rat models [260,261]. Upon dehydration or salt loading in rat models, cleaved active OASIS is observed binding the AVP promoter region, directly

upregulating protein expression [260]. In ATF6 $^{-/-}$  murine models subjected to intermittent water deprivation, similar downstream effects were observed, but signalling pathways were not investigated [261]. ER stress-inducing agents palmitate and oxysterol 27-hydroxycholesterol both result in a reduction in leptin (a long-term mediator of energy balance) expression and extracellular concentrations. This has been attributed, by using ChIP analysis and siRNA knockdowns, to the fact that the PERK downstream target CHOP negatively regulates C/EBP $\alpha$ , transcriptionally down-regulating its translation and release [262,263]. UPR activation has been implicated in the hypothalamic and brown adipose tissue response to thyroid hormone triiodothyronine (T3). Elevated T3 levels induce the UPR downstream of AMPK in the ventromedial nucleus of the hypothalamus, resulting in decreased ceramide levels. JNK1 KO revealed that it acts downstream of this AMPK-dependent activation, possibly as a target of IRE1 but to our knowledge no studies have yet confirmed this [264]. In response to ER stress in hepatocytes, CREBH is exported from the ER and cleaved in the Golgi apparatus. The CREBH cytosolic fragment binds to the promoter region of hepcidin and transcriptionally upregulates its production [265]. These examples of UPR-regulated hormone production and release give scope for further investigation into the longer term, system wide effects of UPR signalling outside of the current focuses on cytotoxicity and acute diseases.

### Physiological ER stress signalling

It has been established that ER stress signalling is important in interorganelle and intercellular interactions. It therefore comes as no surprise that it forms a significant network of interactions upon which normal physiology is based. This is not only the case in humans, but is also conserved throughout species and has been an important fact in the design of experimental model organisms to further study ER stress signalling and its role in physiology and disease.

### Embryology and development

The UPR as the major conduit of ER stress regulation has been extensively studied in developmental biology in the majority of organisms commonly used in translational research. The use of multiple models has been important in discerning the variable ER stress signalling between species, as demonstrated by the discovery that protein quality control in mammals is critically dependent on ATF6 while the major player in

*Caenorhabditis elegans* and *Drosophila melanogaster* is IRE1 [182,266]. Mammalian and other embryos implanted *in vitro* or naturally, undergo a multitude of physical, biochemical and cellular stresses involving epigenetic changes as well as a disproportional increase in protein synthesis load that affect cell differentiation, proliferation and growth.[267]. In zebrafish, transgenic models have been generated to monitor XBP1 splicing during development and implantation, showing that maternal XBP1s is active in oocytes, fertilized eggs and early stage embryos, presenting a potential model for study of the impact of water pollutants on embryogenesis [268]. It was recently shown that in medaka fish the JNK and RIDD pathways are dispensable for growth, with development solely dependent on the XBP1 arm of IRE1 signalling, thereby supporting the hypothesis that XBP1 and RIDD may be differentially utilized in development and homeostasis [269]. In *C. elegans* it has been postulated that the IRE1-XBP1 axis as well as the PERK pathway are responsible for the maintenance of cellular homeostasis during larval development [270]. Pronephros formation was shown to be BiP dependent in *Xenopus* embryos, where BiP morpholino knock-down not only blocked pronephros formation but also attenuated retinoic acid signalling, impacting markers such as the Lim homeobox protein [271]. In early mouse development, it was shown that the BiP promoter is activated in both the trophoectoderm and inner cell mass at embryonic day 3.5 and that absence of BiP leads to proliferative defects and inner cell mass apoptosis, suggesting it is necessary for embryonic cell growth and pluripotent cell survival [272]. Furthermore, mouse studies revealed that ER stress proteins such as BiP, GRP94, calreticulin and PDIA3 were downregulated in adult neural tissues compared to embryonic ones, suggesting a pivotal role for ER stress signalling in the development of neural tissues such as the brain and retina [273]. Beyond the nervous system, ER stress signalling impairment has repeatedly shown mouse embryonic lethality and, in particular in the hepatocellular system, multiple studies have demonstrated that IRE1 and XBP1 signalling defects lead to fetal liver hypoplasia, intrauterine anaemia and early antenatal pancreatic dysfunction [274]. The UPR is intrinsically linked to the mouse embryonic morula–blastocyst transition [275] and this, in combination with evidence that there is an immediate postnatal downregulation of BiP, shows that there is an important role for the UPR both in early and late gestation [276]. Taking all this evidence into consideration, it is apparent that the correct integration of signals both intracellularly and between the developing oocyte, follicular environment and supporting cumulus cells is absolutely essential for embryonic

development, making ER stress signalling a key regulator in the earliest stages of life in all organisms [277].

### Growth and differentiation

Many cell types experience a high protein load during various stages of differentiation and maturation, resulting in ER stress. In several cases, morphological changes required for the final function of the cell would not be possible without transient activation of the UPR's cytoprotective mechanisms. Deletion of PERK in murine models results in loss of pancreatic  $\beta$  cell architecture but not in cell death, and was accompanied by an increase in  $\beta$  cell proliferation. This morphological change results in a diabetes mellitus-like pathology and is not a result of increased cell death as previously proposed [278]. Various haematopoietic lineages require the activation of the UPR in order to survive ER stress resulting from production of immunoglobulins and lysosomal compartments in order to reach maturity [279–281]. One physiological function that is indispensable for survival is the innate immune response, and cell differentiation is at its epicentre. The conversion of B lymphocytes to highly secretory plasma cells is accompanied by a huge expansion of the ER compartment, and genetic alterations to induce immunoglobulin production are good examples of the necessity of ER signalling in normal physiology [123]. This is supported by a study that suggests the UPR, and the PERK pathway in particular, govern the integrity of the haematopoietic stem-cell pool during stress to prevent loss of function [282]. The ability of skin fibroblasts to produce collagens and matrix metalloproteinases (proteins increased at wound sites), along with their ability to differentiate into myofibroblasts, provides another example where physiological ER stress may drive morphological cellular transition [283]. Although not yet fully characterized, the RIDD pathway has been linked to a multitude of physiological processes including lysosomal degradation and xenobiotic metabolism through cytochrome P450 regulation [284]. At the same time, substrates of regulated intramembrane proteolysis such as CREBH are involved in normal physiological processes such as gluconeogenesis [284]. Another substrate of regulated intramembrane proteolysis, OASIS, is involved in multiple stages of bone homeostasis and development. Mice lacking OASIS present with severe osteopenia, which is compounded by the fact that the gene for type I collagen is an OASIS target [285]. Moreover, osteoblast OASIS expression is controlled by factors essential to osteogenesis (BMP2), pointing to a PERK-eIF2 $\alpha$ -ATF4 pathway upregulation during osteoblast

differentiation, where ATF4 restores deficiencies of PERK null osteoblasts all the while impacting apoptosis for bone remodelling [251,286]. Furthermore, a link between osteoblast differentiation and hypoxia has been established, with decreased vascularization shown in OASIS null mice pointing towards a potential role of ER stress in angiogenesis during bone development [287]. This signalling cascade does not only restrict itself to the normal physiology of bone but also modulates UPR signalling in astrocytes and is responsible for the terminal, early to mature, goblet cell differentiation in the large intestine [288–290].

### Metabolism

The ER is a site of significant metabolic regulation. The UPR plays a major role in the regulation of glycolysis and it was recently shown that IRE1 mediates a metabolic decrease upon glucose shortage in neurons, suggesting an important role for the UPR as an adaptive response mechanism in relation to energy metabolism [291]. Moreover, mTOR signalling adjusts global protein synthesis, which is a highly energy consuming process, and thereby regulates energy metabolism (reviewed in [292]).

### Lipid homeostasis

The ER is heavily involved in lipid homeostasis. Characteristically, hepatocytes are enriched in SER, because in addition to protein synthesis, these cells also synthesize bile acids, cholesterol and phospholipids. XBP1 ablation in murine liver results in hypolipidaemia due to feedback activation of IRE1 caused by the lack of XBP1. Activated IRE1 induces the degradation of mRNAs of a cohort of lipid metabolism genes via RIDD, demonstrating the critical role of IRE1–XBP1 signalling in lipid metabolism and suggesting that targeting XBP1 may be a viable approach to the treatment of dyslipidaemias [113]. It was also reported that in hepatocyte-specific IRE1-null mice, XBP1 is involved in very low-density lipoprotein synthesis and secretion [215]. Interestingly, ATF6 has also been shown to have a role in adipogenesis by inducing adipogenic genes and lipid accumulation [293].

### Glucose metabolism

The UPR is also involved in regulating glucose metabolism. Initial murine studies suggested the PERK–eIF2 $\alpha$  arm was responsible for impaired insulin signalling due to knock out effects on beta cells during development. Further studies have since shown that

IRE1 RIDD activity is responsible for a reduction in the mRNA of proinsulin processing proteins, including *INS1*, *PC1* and *SYP*. These effects can be observed in cases of XBP1 deficiency and in cases of extensive UPR activation, highlighting the divergent effects of IRE1 RNase activity [119,221,294].

### Amino acid metabolism

The UPR is also described to be involved in amino acid metabolism. It was recently described that ATF4 mediates increased amino acid uptake upon glutamine deprivation [295]. Furthermore, a low protein diet leads to the upregulation of cytokines mediated by IRE1 and RIG1 which results in an anticancer immune response in tumours [296]. In summary, these findings show the importance of the various UPR arms in cell metabolism and energy homeostasis with effects not only on the cell itself but also on the whole cellular environment.

### Pharmacological targeting of the UPR

Several small molecules have been reported to modulate (activate or inhibit) one or more arms of the UPR. Importantly, these molecules have shown promising beneficial effects in diverse human diseases (Table 1). X-ray cocrystal structures are now available for IRE1 and PERK with several endogenous or exogenous ligands. The understanding of how small molecules bind to the active sites and modulate the function of IRE1 and PERK will have a profound impact on the structure-based drug discovery of novel UPR modulators. Available X-ray structures, in addition to mutagenesis analysis of critical amino acids [297], have revealed a variety of unexpected allosteric binding sites on IRE1 [297–299].

### Pharmacological modulators of IRE1

IRE1 signalling information along with CHOP/Gal4-Luc cells and UPR-Luc engineered cells were used to screen large chemical libraries in high throughput screening assays for discovery of pathway-selective modulators of IRE1 [300].

### IRE1 ATP-binding site

IRE1 modulators have been discovered primarily by traditional drug discovery methods, identifying inhibitors specific to the kinase or RNase domain (Table 1). The IRE1 kinase modulators were used as tools to understand the allosteric relationship between the



**Table 1.** Different modulators that target the UPR-transducer protein pathways. Molecule name, respective molecular target and brief description with the associated reference are provided (ND: not determined).

UPR Arm	Name	Target	Brief description	Reference
PERK	GSK2656157	PERK Kinase	In preclinical stage for multiple myeloma and pancreatic cancer	[314,364]
	Salubrinal	GADD34/PP1c	Inhibition of eIF2 $\alpha$ dephosphorylation In ALS, it increases lifespan of mutant superoxide dismutase 1 transgenic mice In Parkinson's disease, it increases neuronal survival of $\alpha$ -synuclein transgenic mice	[365–367]
	ISRIB	eIF2 $\beta$	Decreased ATF4 expression	[322]
	Guanabenz Sephin1	GADD34/PP1c GADD34 (PP1c)	Inhibitor of eIF2 $\alpha$ phosphatase, Inhibitor of eIF2 $\alpha$ phosphatase	[368] [369]
IRE1	Salicylaldimines	IRE1 RNase	IRE1 $\alpha$ RNase active-site inhibitor	[305]
	STF-083010	IRE1 RNase	IRE1 $\alpha$ RNase active-site inhibitor	[308]
	MKC-3946	IRE1 RNase	IRE1 $\alpha$ RNase active-site inhibitor In preclinical stage for multiple myeloma treatment	[307,370]
	4 $\mu$ 8c	IRE1 RNase	IRE1 $\alpha$ RNase active-site inhibitor In preclinical stage for multiple myeloma treatment	[306]
	APY29	IRE1 Kinase	IRE1 $\alpha$ kinase active-site inhibitor	[303]
	Sunitinib	IRE1 Kinase	IRE1 $\alpha$ kinase active-site inhibitor FDA approved for renal cell carcinoma It acts on multiple kinases	[85,304]
	KIRA	IRE1 Kinase	IRE1 $\alpha$ kinase active-site inhibitor	[371]
	Toyocamycin	IRE1 RNase	IRE1 $\alpha$ RNase active-site inhibitor In preclinical stage for various cancers treatment	[309,372]
	3-ethoxy-5,6-dibromosalicylaldehyde	IRE1 RNase	IRE1 $\alpha$ RNase active site inhibitor	[305]
	Apigenin FIRE peptide	Proteasome IRE1 Kinase	Increase of IRE1a nuclease activity in model Modulation IRE1 oligomerization <i>in vitro</i> , Xbp1 mRNA cleavage <i>in vitro</i> , in cell culture and <i>in vivo</i> ( <i>Caenorhabditis elegans</i> )	[373] [85]
ATF6	Apigenin	ATF6	Upregulation of ATF6 expression	[373]
	Baicalein	ATF6	Upregulation of ATF6 expression	[374]
	Ceapin	ND	Inhibitor of ATF6	[323]
	Kaempferol	ATF6	Downregulation of ATF6 expression	[375]
	Melatonin	ATF6	Inhibitor of ATF6	[325]
	Compound 147	ATF6	Activator of ATF6	[376]
	Compound 263	ATF6	Activator of ATF6	[376]
	16F16	PDI	Inhibitor of PDI	[377]

kinase and RNase domains [301,302]. Kinase inhibitors can be broadly classed as (a) ATP-competitive inhibitors that inhibit the kinase domain and activate the RNase domain and (b) ATP-competitive inhibitors that inhibit the kinase domain and inactivate RNase (kinase inhibiting RNase attenuators – KIRAs). Available IRE1 crystal structures reveal a possible mechanism of RNase activation by conformational changes that occur in the kinase domain when transitioning from a monomeric to an active dimeric state. Type I IRE1 kinase inhibitors include APY29 [303] and sunitinib [304], which target the ATP-binding site and

inhibit the phosphorylation but stabilize the active form of the kinase domain. An active kinase conformation is seen in human *apo* dP-IRE1\* (PDB 5HG1), as a back-to-back dimer. Notably, the DFG motif (Asp711-Phe712-Gly713) faces into the active site (DFG-in), with helix- $\alpha$ C-in conformation. In contrast, human IRE1 bound to KIRA compound 33 (PDB: 4U6R) shows an inactive kinase conformation, with DFG-in and helix- $\alpha$ C-out conformation. The inactive conformation is incompatible with back-to-back dimer formation due to the displaced helix- $\alpha$ C [301]. Imidazopyrazine-based inhibitors and other KIRAs

allosterically inhibit the RNase activity of phosphorylated IRE1 by possibly displacing helix- $\alpha$ C from an active conformation to an inactive conformation [301].

### IRE1 RNase-binding site

IRE1 RNase inhibitors include salicylaldehydes [305] 4 $\mu$ 8C [306], MKC-946 [307], STF-83010 [308], toyocamycin [309] and hydroxyl-aryl-aldehydes [86]. The reported cocrystal structures of murine IRE1 $\alpha$  with salicylaldehyde-based inhibitor show that Lys 907 is involved in Schiff base arrangement (PDB code: 4PL3 [86]). Lys 907 is a crucial residue present within the hydrophobic pocket of the IRE1 RNase catalytic site [310]. Quercetin is reported to activate IRE1 through a site distinct from the nucleotide-binding site (crystal structure PDB 3LJ0), increasing the population of IRE1 dimers *in vitro* [299]. A recent *in silico* study identified the anthracycline antibiotic doxorubicin as an inhibitor of the IRE1-XBP1 axis [311]. Covalent binders are very efficient in the sense that they completely block the proteins to which they bind, but this can also have several drawbacks [312]. Noncovalent kinase and allosteric modulators in general inhibit competitively and are thus less efficient, but can at the same time be extremely useful in obtaining new insights for developing selective and potent modulators of IRE1 $\alpha$ -XBP1 signalling (Table 1).

### Other IRE1 modulators

Peptides derived from the kinase domain of human IRE1 promote oligomerization *in vitro*, enhancing XBP1 mRNA cleavage activity *in vitro* and *in vivo* [85]. However, although peptide-based modulators have limited clinical application [313] (Table 1) peptide mimetics may prove more useful. These are different aspects that can be exploited to develop selective IRE1 modulators. Despite significant progress in understanding IRE1 signalling and in the development of modulators of IRE1 activity, several questions still remain to be answered to fully control IRE1 activity and signalling outcomes, including how to selectively target the XBP1 and RIDD arms of IRE1 signalling.

### Pharmacological modulators of PERK

Through biochemical screening of exclusive library collections and structure-based lead optimization, GSK discovered PERK inhibitors GSK2606414 and GSK2656157 [314]. These potent PERK inhibitors can be orally administered [314], reducing tumour growth in mouse xenograft models [314,315]. GSK2606414

was also the first oral small molecule to prevent neurodegeneration *in vivo* in prion-diseased mice, with GSK2606414 reducing the levels of p-PERK and p-eIF2 $\alpha$  and restoring protein synthesis rates [316]. Despite the promising selectivity profile, pharmacological inhibition of PERK in mice caused damage to exocrine cells and pancreatic beta cells, a similar phenotype to that observed in PERK<sup>-/-</sup> mice [317]. Furthermore, GSK2606414 and GSK2656157 were found recently to inhibit RIPK1 at nanomolar concentrations [318]. To overcome the  $\beta$ -cell toxicity, small molecules modulating the eIF2 $\alpha$  pathway without directly inhibiting PERK were examined. Integrated stress response inhibitor (ISRIB) is the first small molecule described to bind and activate guanine nucleotide exchange factor eIF2B [319,320]. Unlike GSK inhibitors, ISRIB did not show any pancreatic toxicity [321]. Interestingly, ISRIB increased learning and memory in WT mice [322] (Table 1).

### ATF6 modulators

The identification of small molecules that modulate ATF6 has been challenging due to lack of potentially druggable binding sites and unavailability of the protein crystal structure. Recently, Walter and colleagues identified selective inhibitors of ATF6 signalling, the small molecules Ceapins, using a high throughput cell-based screen [323]. Ceapins do not affect the IRE1 and PERK arms of the UPR. Ceapins are chemically classed as pyrazole amides and extensive biochemical and cell biology evidence show that they trap ATF6 in the ER and thus prevent its translocation to the Golgi upon stress [324]. Ceapins sensitize cells to ER stress without affecting unstressed cells and hence have potential to be developed within the framework of a therapeutic strategy to induce cell death in cancer cells. A recent study identified melatonin as an ATF6 inhibitor, leading to enhanced liver cancer cell apoptosis through decreased COX-2 expression [325]. The activation of ATF6 depends on a redox process involving PDIs suggesting that PDI inhibitors such as PACMA31 [326], RB-11-ca [327], P1 [327] and 16F16 [328] may be able to modulate ATF6 activation. Additionally, the serine protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride is reported to prevent ER stress-induced cleavage of ATF6 [329] (Table 1). Albeit the above developments hold strong promise for the future, very little is known to date about specific binding sites, which together with the lack of a crystal structure and insufficient templates to enable homology modelling, rational drug design targeting ATF6 remains a

challenge. Availability of an ATF6 crystal structure is in this sense the key aspect, as this will provide atomistic level understanding of interactions and mechanism of action, and enable *in silico* based rational design of ATF6 modulators.

## The UPR in the clinic

In this section, we review recent preclinical and clinical studies in which UPR components were used as disease biomarkers or as therapeutic targets (Fig. 3). As already described in section Perturbing ER functions molecules have been designed to modulate ER stress by inducing the UPR (Brefeldin A, DTT), inhibiting SERCA  $\text{Ca}^{2+}$  ATPases (thapsigargin) or preventing the generation of glycoproteins, and hence, the induction of ER stress through calcium imbalance or misfolded protein accumulation. They were touted as potential antitumour therapies as they could potentially induce tumour cell death through ER stress over-activation. However, none of these compounds were used in the clinic due to their lack of specificity and high toxicity. It has been reported though that a pro-drug analogue of thapsigargin, mipsagargin, did display acceptable tolerability and favourable pharmacokinetic profiles in patients with solid tumours [330]. On the other hand, section 6 describes molecules that inhibit the various arms of the UPR.

## UPR biomarkers

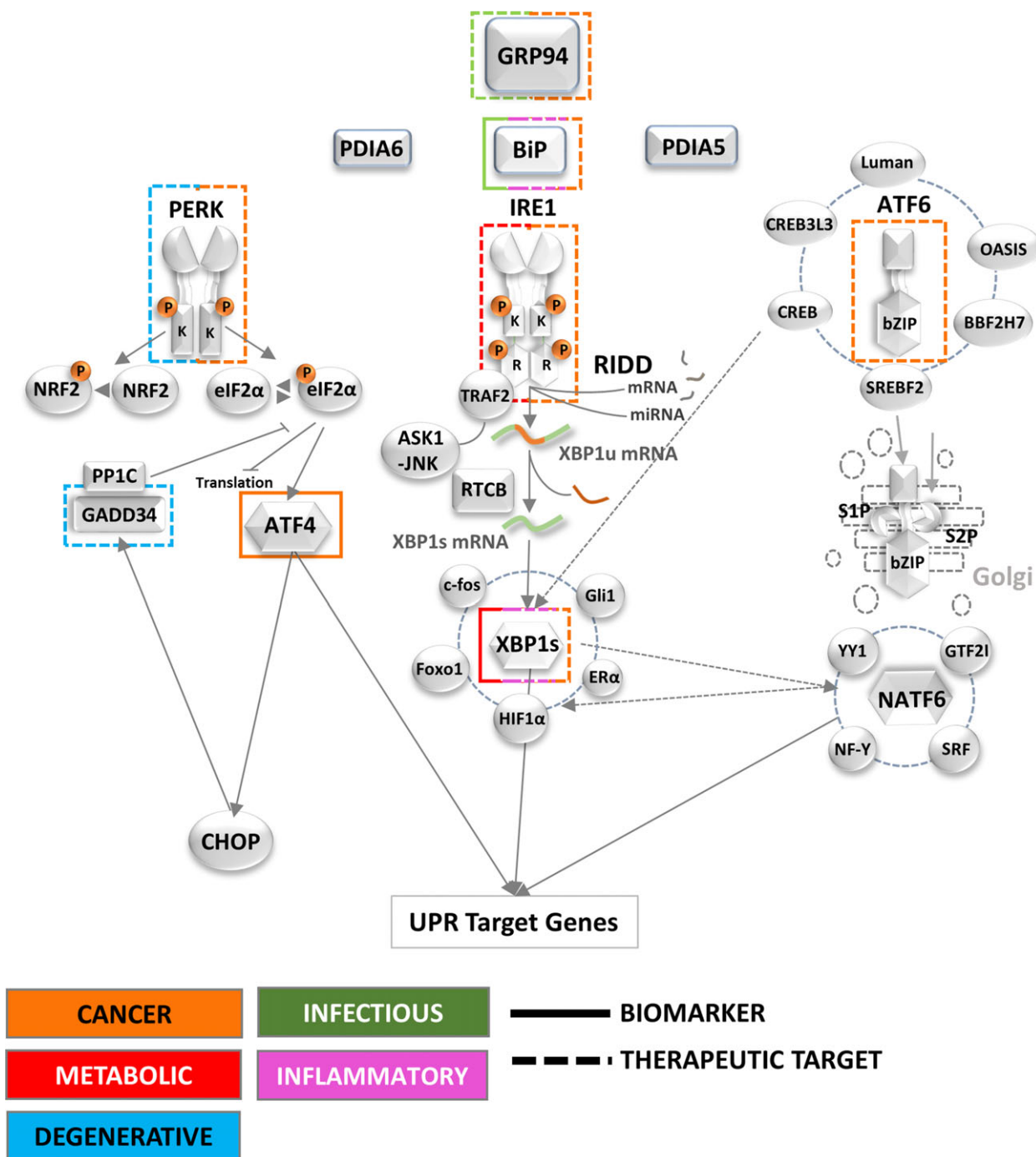
Changes in UPR and ER stress markers in blood or tissue biopsy samples can be indicative of disease state and could be/are utilized as valuable biomarkers for different human pathologies. For instance, BiP has strong immunological reactivity when released into the extracellular environment [331], and in 1993, it was the first ER stress protein associated with the pathogenesis of osteogenesis imperfecta [332]. Since then, further evidence suggests overexpression of BiP in several human diseases (reviewed in [333]). The UPR transcription factors can also be seen as potential biomarkers of various diseases. ATF4 is upregulated and contributes to progression and metastasis in patients with oesophageal squamous cell carcinoma [334]. Similarly, XBP1 overexpression is linked to progressive clinical stages and degree of tumour malignancy in osteosarcoma [335]. In contrast, IRE1–XBP1 downregulation can differentiate germinal centre B cell-like lymphoma from other diffuse large B-cell lymphoma subtypes and contributes to tumour growth [336]. Moreover, XBP1 is genetically linked to inflammatory bowel disease (IBD) [337]. Using cohorts of IBD

patients to test the association of 20 SNPs across the XBP1 gene region, it was found that three SNPs rs5997391, rs5762795 and rs35873774 are associated with disease, thus linking cell-specific ER stress changes with the induction of organ-specific inflammation. Quantitative changes in ER stress chaperones in the CSF have been proposed as possible biomarkers to monitor the progression of neurodegenerative diseases such as ALS [338,339]. Finally, the mesencephalic astrocyte-derived neurotrophic factor (MANF) can be used as a urine biomarker for ER stress-related kidney diseases [340]. MANF localizes in the ER lumen and is secreted in response to ER stress in several cell types. Similarly, angiogenin was identified as an ER stress responsive biomarker found in the urine of patients with kidney damage [341]. Thus, noninvasive ER stress-related biomarkers can be used to stratify disease risk and disease development (Fig. 3).

## ER stress and UPR-based therapies

Beyond their use as biomarkers, ER stress signalling components also represent relevant therapeutic targets. BiP was recently recognized as a universal therapeutic target for human diseases such as cancer and bacterial/viral infections [333]. Antibodies targeting BiP exhibited antitumoural activity and enhanced radiation efficacy in non-small-cell lung cancer and glioblastoma multiforme in mouse xenograft models [342]. It was also shown that short-term systemic treatment with a monoclonal antibody against BiP suppressed AKT activation and increased apoptosis in mice with endometrial adenocarcinoma [343]. Moreover, the ER-resident GRP94 is being evaluated as a therapeutic target because of its ability to associate with cellular peptides irrespective of size or sequence [344]. Preclinical studies have linked GRP94 expression to cancer progression in multiple myeloma, hepatocellular carcinoma, breast cancer and colon cancer. Finally, this protein has been identified as a strong modulator of the immune system that could be used in anticancer immunotherapy [345].

ER stress-induced transcription factors can also represent relevant targets. Thus, XBP1s has been one of the main targets for drug discovery and gene therapy [346]. Elimination of XBP1 improves hepatosteatosis, liver damage and hypercholesterolaemia in animal models. As such direct targeting of IRE1 or XBP1 can be a possible strategy to treat dyslipidaemias [113]. In cancer, toyocamycin was shown to inhibit the constitutive activation of XBP1s expression in multiple myeloma cells as well as in patient primary samples [309]. Despite being the least studied UPR arm, there are



**Fig. 3.** UPR disease biomarkers and therapeutic targets. Schematic representation of the UPR signalling pathway as defined in Fig. 2 and annotated with the relevance to disease of each component. The colour code indicates the type of disease (cancer: orange; metabolic disease: red; degenerative disease: blue; infectious disease: green; inflammatory disease: pink) and the lines indicate the role as biomarker (continuous line) or therapeutic target (dashed line).

instances that ATF6 can be a specific clinical target. The activation of ATF6 but not IRE1 or PERK has been linked with airway remodelling in a mouse model of asthma [347]. Additionally, these studies showed

that expression of orosomucoid-like 3 (ORMDL3) regulates ATF6 expression and airway remodelling through ATF6 target genes such as SERCA2b, TGFβ1, ADAM8 and MMP9 (Fig. 3, Table 2).

ER stress targets are also strong candidates for immunotherapy and vaccines development, a good example of which is the production of chaperone protein-based cancer vaccines termed chaperone-rich cell lysate (CRCL) [348]. The CRCL are purified from tumour tissue or recombinantly produced and applied as vaccines against murine and canine cancers or infectious diseases. Advantages of CRCL vaccines include small quantities and easily obtained starting materials [349]. Furthermore, DNA vaccination with gp96-peptide fusion proteins showed increased resistance against the intracellular bacterial pathogen *Listeria monocytogenes* in a mouse model [350]. To improve the efficacy of gp96 vaccines, gp96 was pooled with CpG in combination with anti-B7H1 or anti-interleukin-10 monoclonal antibodies to treat mice with large tumours [351]. The heterogeneous or allogeneic gp96 vaccines protected mice from tumour challenge and re-challenge. In addition to its role as a molecular chaperone, GRP94 was likewise identified as a peptide carrier for T-cell immunization [352]. However, the immunological application of GRP94 derived from its peptide binding capacity was not further investigated (Fig. 3, Table 2). The activation of ER stress has been

reported as well in different critical care diseases models, such as sepsis [331,332], liver, heart, brain and kidney ischaemia [353–359] and haemorrhagic shock [334,335]. But, the pathophysiological impact of ER stress activation in these conditions severely lacks characterization. Multiple factors such as inflammation, hypoxia present in sepsis and shock can induce ER stress but its effects are ambivalent. It has been shown that induction of ER stress is cytoprotective [353,354], and that proteostasis promoters/disruptors such as 4-PBA [336] or TUDCA [337] can be used to improve disease outcome. The increase of CHOP in renal tissue was reported to inhibit inflammatory response in and provide protection against kidney injury [336]. Moreover, the activation of PERK seems to facilitate survival of lipopolysaccharide-treated cardiomyocytes by promoting autophagy [338]. Additionally, the activation of ATF6 before ischaemia reduced myocardial tissue damage during ischaemia/reperfusion (I/R) injury [339]. Furthermore, induction of BiP in cardiomyocytes stimulated AKT signalling and protected against oxidative stress, conferring cellular I/R damage protection [340]. In contrast, inhibition of ER stress was indicated to limit cellular damage in

**Table 2.** ER stress-centred clinical trials. A range of clinical entities in endocrinology, oncology and paediatrics have been targeted through clinical trials. This table presents such trials detailing the trial targeted, interventional agent investigated and national authority carrying out the investigation.

Trial	Disease	Intervention	Country
Role of ER stress in the pathophysiology of type 2 diabetes	<ul style="list-style-type: none"> <li>Diabetes mellitus, type 2</li> </ul>	No intervention	France
ER stress and resistance to treatments in Ph-negative myeloproliferative neoplasms	<ul style="list-style-type: none"> <li>Polycythemia vera</li> <li>Essential thrombocythemia</li> </ul>	Biological: RNA sample of total leucocytes before start of treatment	France
Effect of ER stress on metabolic function	<ul style="list-style-type: none"> <li>Insulin resistance</li> <li>Diabetes</li> <li>Obesity</li> </ul>	Drug: TUDCA Other: placebo Drug: sodium phenylbutyrate	United States
ER stress in chronic respiratory diseases	<ul style="list-style-type: none"> <li>Chronic airway disorders</li> <li>Lung cancer</li> </ul>	Observational	South Korea
TUDCA for protease-inhibitor associated insulin resistance	<ul style="list-style-type: none"> <li>HIV-related insulin resistance</li> <li>Protease inhibitor-related Insulin resistance</li> </ul>	Drug: TUDCA Other: placebo tablet	United States
ER stress in NAFLD	<ul style="list-style-type: none"> <li>Obesity</li> <li>NAFLD</li> </ul>	Drug: methyl-D9-choline	United States
TUDCA in new-onset type 1 diabetes	<ul style="list-style-type: none"> <li>Type 1 diabetes</li> </ul>	Drug: TUDCA Drug: Sugar Pill (placebo)	United States
Effects of Liraglutide on ER stress in obese patients with type 2 diabetes	<ul style="list-style-type: none"> <li>Type 2 diabetes</li> </ul>	Drug: liraglutide	United States
A clinical trial of dantrolene sodium in paediatric and adult patients with wolfram syndrome	<ul style="list-style-type: none"> <li>Wolfram syndrome</li> <li>Diabetes mellitus</li> <li>optic nerve atrophy</li> <li>Ataxia</li> </ul>	Drug: dantrolene sodium	United States

**Box 1. First-in-human trial**

Intravenous infusion of GRP78/BiP is safe in patients. In 2006, Brownlie *et al.* [362] reported that the prophylactic or therapeutic parenteral delivery of BiP ameliorates clinical and histological signs of inflammatory arthritis in mice. Ten years later, the first human clinical trial using intravenous BiP demonstrated that GRP78/BiP is safe in patients with active rheumatoid arthritis and some patients had clinical and biological improvements [363]. In phase I/IIA RAGULA trial, 42 patients were screened, and 24 were randomized to receive either BiP or placebo. The study showed that after a single intravenous infusion, BiP may induce remission lasting up to 3 months in rheumatoid arthritis patients.

pathologies such as hepatic I/R [341]. This contradiction may be due to interference between UPR and inflammatory pathways. CHOP<sup>-/-</sup> mice were reported to have more prominent increase in NF- $\kappa$ B activation and further upregulation of proinflammatory genes (CXCL-1, MIP-2, IL-6) [342]. Interestingly, inhibition of IRE1-NF- $\kappa$ B by resveratrol protected against sepsis-induced kidney failure [343]. In this light, the modulation of specific UPR branches is promising approach for therapy of critical care diseases.

As discussed above, understanding and characterizing the UPR has provided several potential targets to develop new therapeutics for various diseases, with an encouraging increase in the number of clinical trials based on ER stress pathway targets or associated drugs. Several of these trials [ClinicalTrials.gov, European Clinical Trials Database and the ISRCTN registry] have focused on diabetes mellitus. A trial testing TUDCA and 4-PBA for the treatment of high lipid levels or insulin resistance was conducted by the Washington University School of Medicine; however, although this study was completed in 2014, the findings are not available yet. The results from the first completed human trial using BiP for rheumatoid arthritis are described in Box 1. We can anticipate that clinical trials to test ER stress targeting drugs in several other diseases will shortly ensue.

**Concluding remarks**

The ER has evolved in our knowledge from a key player in proteostasis and the secretory pathway to a cornerstone of metabolic functions. Such wealth of information has allowed the identification of numerous

mechanisms for fine-tuning ER signalling, as well as motivated the need for their better characterization towards relevant health-related applications. This drive to further ER knowledge has also led to the identification of emerging roles for the ER in physiology and disease. In particular, it appears an indispensable tool for cellular communication that reaches beyond the intracellular space. The concept of transmissible ER stress illustrates the far-reaching control that ER signalling exerts in interorgan communication affecting disease pathogenesis and normal physiology [360,361].

Our increasing knowledge of ER signalling mechanisms presents opportunities to exploit the resulting applications on multiple fronts, including bioengineering and health, concepts that may routinely overlap. For example, boosting ER protein production capacity may be applied to cell engineering to increase biologic therapy production. This will drive down costs of biologics, helping demand to be met and leading to more widely available medications, thus having a significant effect on public health. Population-wide consequences of ER modulation may not be restricted to the production of biologic therapies as its applications could also contribute to bioengineering approaches for crop or livestock improvement.

A thorough understanding of the ER stress response and its role in physiology and pathophysiology can be applied to develop new ER stress targeted therapies and stratifying patients into cohorts suitable for ER-targeted therapies. Considering the enormity of attrition rates of novel therapeutic discovery in an ever-tightening financial climate, there is an urgent need for new therapeutic targets as well as precision tools that target and guide innovation to specific patient pools. ER stress signalling may provide such tools. Not only is it central to life itself but it is involved in a wide array of clinical presentations. Moreover, its effect on heterogeneous presentations within the same diseases makes it an attractive target for translational precision medicine. Of course, when undertaking medical research or trying to solve a biomolecular functional mystery one cannot look past the logistical aspect of the task ahead. The conserved metazoan nature of ER stress signalling combined with the emergence of high throughput and *in silico* strategies supplies researchers with a wealth of tools to study pathophysiology, from structure to function in multiple *in vivo* and *in vitro* models, producing robust results to be put forward for clinical scrutiny while all the while observing both the safeguards of the declaration of Helsinki and ethics on animal experimentation. Our deeper understanding of the ER and its major homeostatic regulator, the UPR response, is introducing an individualized molecular

approach to health management at a preventative, diagnostic and therapeutic level and, uncovering the genetic architecture underlying the ER stress response could significantly influence future therapeutic strategies in patients.

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## References

- Porter KR, Claude A & Fullam EF (1945) A study of tissue culture cells by electron microscopy: methods and preliminary observations. *J Exp Med* **81**, 233–246.
- Palade GE & Porter KR (1954) Studies on the endoplasmic reticulum. I. Its identification in cells *in situ*. *J Exp Med* **100**, 641–656.
- Alberts B, Johnson A, Lewis J, Raff M, Roberts K & Walter P (2002) *Molecular Biology of the Cell*, 4th edn. Garland Science, New York, NY.
- Borgese N, Francolini M & Snapp E (2006) Endoplasmic reticulum architecture: structures in flux. *Curr Opin Cell Biol* **18**, 358–364.
- Shibata Y, Voeltz GK & Rapoport TA (2006) Rough sheets and smooth tubules. *Cell* **126**, 435–439.
- Hayashi T, Rizzuto R, Hajnoczky G & Su TP (2009) MAM: more than just a housekeeper. *Trends Cell Biol* **19**, 81–88.
- Toulmay A & Prinz WA (2011) Lipid transfer and signaling at organelle contact sites: the tip of the iceberg. *Curr Opin Cell Biol* **23**, 458–463.
- Daste F, Galli T & Taresté D (2015) Structure and function of longin SNAREs. *J Cell Sci* **128**, 4263–4272.
- Rowland AA, Chitwood PJ, Phillips MJ & Voeltz GK (2014) ER contact sites define the position and timing of endosome fission. *Cell* **159**, 1027–1041.
- Wilhelm LP, Tomasetto C & Alpy F (2016) Touché! STARD3 and STARD3NL tether the ER to endosomes. *Biochem Soc Trans* **44**, 493–498.
- Wilhelm LP, Wendling C, Védie B, Kobayashi T, Chenard M, Tomasetto C, Drin G & Alpy F (2017) STARD3 mediates endoplasmic reticulum-to-endosome cholesterol transport at membrane contact sites. *EMBO J* **36**, 1412–1433.
- Henne WM, Zhu L, Balogi Z, Stefan C, Pleiss JA & Emr SD (2015) Mdm1/Snx13 is a novel ER-endolysosomal interorganelle tethering protein. *J Cell Biol* **210**, 541–551.
- Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T & Yamamoto A (2010) Electron tomography reveals the endoplasmic reticulum as a membrane source for autophagosome formation. *Autophagy* **6**, 301–303.
- Uemura T, Yamamoto M, Kametaka A, Sou Y, Yabashi A, Yamada A, Annoh H, Kametaka S, Komatsu M & Waguri S (2014) A cluster of thin tubular structures mediates transformation of the endoplasmic reticulum to autophagic isolation membrane. *Mol Cell Biol* **34**, 1695–1706.
- Stefan CJ, Manford AG, Baird D, Yamada-Hanff J, Mao Y & Emr SD (2011) Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. *Cell* **144**, 389–401.
- Braakman I & Balleid NJ (2011) Protein folding and modification in the mammalian endoplasmic reticulum. *Annu Rev Biochem* **80**, 71–99.
- Hebert DN & Molinari M (2007) In and out of the ER: protein folding, quality control, degradation, and related human diseases. *Physiol Rev* **87**, 1377–1408.
- Wallis AK & Freedman RB (2013) Assisting oxidative protein folding: how do protein disulphide-isomerases couple conformational and chemical processes in protein folding? *Top Curr Chem* **328**, 1–34.
- Aebi M, Bernasconi R, Clerc S & Molinari M (2010) N-glycan structures: recognition and processing in the ER. *Trends Biochem Sci* **35**, 74–82.
- Meusser B, Hirsch C, Jarosch E & Sommer T (2005) ERAD: the long road to destruction. *Nat Cell Biol* **7**, 766–772.
- Hebert DN, Bernasconi R & Molinari M (2010) ERAD substrates: which way out? *Semin Cell Dev Biol* **21**, 526–532.
- Kobayashi T, Tanaka K, Inoue K & Kakizuka A (2002) Functional ATPase activity of p97/valosin-containing protein (VCP) is required for the quality control of endoplasmic reticulum in neuronally differentiated mammalian PC12 cells. *J Biol Chem* **277**, 47358–47365.
- Enns GM, Shashi V, Bainbridge M, Gambello MJ, Zahir FR, Bast T, Crimian R, Schoch K, Platt J, Cox R *et al.* (2014) Mutations in NGLY1 cause an inherited disorder of the endoplasmic reticulum-associated degradation (ERAD) pathway. *Genet Med* **16**, 751–758.
- Kamhi-Nesher S, Shenkman M, Tolchinsky S, Fromm SV, Ehrlich R & Lederkremer GZ (2001) A novel quality control compartment derived from the endoplasmic reticulum. *Mol Biol Cell* **12**, 1711–1723.
- Huyer G, Longworth GL, Mason DL, Mallampalli MP, McCaffery JM, Wright RL & Michaelis S (2004)

- A striking quality control subcompartment in *Saccharomyces cerevisiae*: the endoplasmic reticulum-associated compartment. *Mol Biol Cell* **15**, 908–921.
- 26 Brown MS & Goldstein JL (1999) A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci USA* **96**, 11041–11048.
- 27 Fagone P & Jackowski S (2009) Membrane phospholipid synthesis and endoplasmic reticulum function. *J Lipid Res* **50**, S311–S316.
- 28 Yen C-LE, Stone SJ, Koliwad S, Harris C & Farese RV (2008) Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *J Lipid Res* **49**, 2283–2301.
- 29 Jo Y & DeBose-Boyd RA (2010) Control of cholesterol synthesis through regulated ER-associated degradation of HMG CoA reductase. *Crit Rev Biochem Mol Biol* **45**, 185–198.
- 30 Patwardhan GA, Beverly LJ & Siskind LJ (2016) Sphingolipids and mitochondrial apoptosis. *J Bioenerg Biomembr* **48**, 153–168.
- 31 Barlowe C, Orci L, Yeung T, Hosobuchi M, Hamamoto S, Salama N, Rexach MF, Ravazzola M, Amherdt M & Schekman R (1994) COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. *Cell* **77**, 895–907.
- 32 Siddiqi S, Saleem U, Abumrad NA, Davidson NO, Storch J, Siddiqi SA & Mansbach CM (2010) A novel multiprotein complex is required to generate the prechylomicron transport vesicle from intestinal ER. *J Lipid Res* **51**, 1918–1928.
- 33 Clapham DE (2007) Calcium signaling. *Cell* **131**, 1047–1058.
- 34 Meldolesi J & Pozzan T (1998) The endoplasmic reticulum Ca<sup>2+</sup> store: a view from the lumen. *Trends Biochem Sci* **23**, 10–14.
- 35 Hetz C (2012) The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol* **13**, 89–102.
- 36 Hanahan D & Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.
- 37 Holderfield M, Deuker MM, McCormick F & McMahon M (2014) Targeting RAF kinases for cancer therapy: BRAF mutated melanoma and beyond. *Nat Rev Cancer* **14**, 455–467.
- 38 Piwocka K, Vejda S, Cotter TG, O'Sullivan GC & McKenna SL (2006) Bcr-Abl reduces endoplasmic reticulum releasable calcium levels by a Bcl-2-independent mechanism and inhibits calcium-dependent apoptotic signaling. *Blood* **107**, 4003–4010.
- 39 Pluquet O, Pourtier A & Abbadie C (2015) The unfolded protein response and cellular senescence. A review in the theme: cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. *Am J Physiol Cell Physiol* **308**, C415–C425.
- 40 Chen H-J, Anagnostou G, Chai A, Withers J, Morris A, Adhikaree J, Pennetta G & de Belleruche JS (2010) Characterization of the properties of a novel mutation in VAPB in familial amyotrophic lateral sclerosis. *J Biol Chem* **285**, 40266–40281.
- 41 Nishimura AL, Mitne-Neto M, Silva HCA, Richieri-Costa A, Middleton S, Cascio D, Kok F, Oliveira JRM, Gillingwater T, Webb J *et al.* (2004) A Mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am J Hum Genet* **75**, 822–831.
- 42 Harding HP & Ron D (2002) Endoplasmic reticulum stress and the development of diabetes: a review. *Diabetes* **51**, S455–S461.
- 43 Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E & Mori M (2002) Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *J Clin Invest* **109**, 525–532.
- 44 Colombo C, Porzio O, Liu M, Massa O, Vasta M, Salardi S, Beccaria L, Monciotti C, Toni S, Pedersen O *et al.* (2008) Seven mutations in the human insulin gene linked to permanent neonatal/infancy-onset diabetes mellitus. *J Clin Invest* **118**, 2148–2156.
- 45 Støy J, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, Below JE, Hayes MG, Cox NJ, Lipkind GM *et al.* (2007) Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci USA* **104**, 15040–15044.
- 46 Giampietri C, Petrunaro S, Conti S, Facchiano A, Filippini A & Ziparo E (2015) Cancer microenvironment and endoplasmic reticulum stress response. *Mediators Inflamm* **2015**, 417281.
- 47 Jin ML, Park SY, Kim YH, Oh J-I, Lee SJ & Park G (2014) The neuroprotective effects of cordycepin inhibit glutamate-induced oxidative and ER stress-associated apoptosis in hippocampal HT22 cells. *Neurotoxicology* **41**, 102–111.
- 48 Stechmann B, Bai S-K, Gobbo E, Lopez R, Merer G, Pinchard S, Panigai L, Tenza D, Raposo G, Beaumelle B *et al.* (2010) Inhibition of retrograde transport protects mice from lethal ricin challenge. *Cell* **141**, 231–242.
- 49 Olden K, Pratt RM, Jaworski C & Yamada KM (1979) Evidence for role of glycoprotein carbohydrates in membrane transport: specific inhibition by tunicamycin. *Proc Natl Acad Sci USA* **76**, 791–795.
- 50 Schultz AM & Oroszlan S (1979) Tunicamycin inhibits glycosylation of precursor polyprotein encoded by env gene of Rauscher murine leukemia virus. *Biochem Biophys Res Commun* **86**, 1206–1213.
- 51 Datema R & Schwarz RT (1979) Interference with glycosylation of glycoproteins. Inhibition of formation



- of lipid-linked oligosaccharides *in vivo*. *Biochem J* **184**, 113–123.
- 52 Cleland WW (1964) Dithiothreitol, a new protective reagent for SH groups. *Biochemistry* **3**, 480–482.
- 53 Liu ES, Ou JH & Lee AS (1992) Brefeldin A as a regulator of grp78 gene expression in mammalian cells. *J Biol Chem* **267**, 7128–7133.
- 54 Thastrup O, Cullen PJ, Drøbak BK, Hanley MR & Dawson AP (1990) Thapsigargin, a tumor promoter, discharges intracellular Ca<sup>2+</sup> stores by specific inhibition of the endoplasmic reticulum Ca<sup>2+</sup>(+)-ATPase. *Proc Natl Acad Sci USA* **87**, 2466–2470.
- 55 Pirot P, Naamane N, Libert F, Magnusson NE, Ørntoft TF, Cardozo AK & Eizirik DL (2007) Global profiling of genes modified by endoplasmic reticulum stress in pancreatic beta cells reveals the early degradation of insulin mRNAs. *Diabetologia* **50**, 1006–1014.
- 56 Malo A, Krüger B, Göke B & Kubisch CH (2013) 4-phenylbutyric acid reduces endoplasmic reticulum stress, trypsin activation, and acinar cell apoptosis while increasing secretion in rat pancreatic acini. *Pancreas* **42**, 92–101.
- 57 Lee YY, Hong SH, Lee YJ, Chung SS, Jung HS, Park SG & Park KS (2010) Tauroursodeoxycholate (TUDCA), chemical chaperone, enhances function of islets by reducing ER stress. *Biochem Biophys Res Commun* **397**, 735–739.
- 58 Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Görgün CZ & Hotamisligil GS (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* **313**, 1137–1140.
- 59 Lepock JR (2005) How do cells respond to their thermal environment? *Int J Hyperthermia* **21**, 681–687.
- 60 Bettaieb A & Averill-Bates DA (2005) Thermotolerance induced at a mild temperature of 40°C protects cells against heat shock-induced apoptosis. *J Cell Physiol* **205**, 47–57.
- 61 Liu Y, Sakamoto H, Adachi M, Zhao S, Ukai W, Hashimoto E, Hareyama M, Ishida T, Imai K & Shinomura Y (2012) Heat stress activates ER stress signals which suppress the heat shock response, an effect occurring preferentially in the cortex in rats. *Mol Biol Rep* **39**, 3987–3993.
- 62 Liu X, Wang M, Chen H, Guo Y, Ma F, Shi F, Bi Y & Li Y (2013) Hypothermia protects the brain from transient global ischemia/reperfusion by attenuating endoplasmic reticulum response-induced apoptosis through CHOP. *PLoS ONE* **8**, e53431.
- 63 Mollereau B (2015) Cooling-induced ER stress is good for your brain. *EBioMedicine* **2**, 482–483.
- 64 Zeeshan H, Lee G, Kim H-R & Chae H-J (2016) Endoplasmic reticulum stress and associated ROS. *Int J Mol Sci* **17**, 327.
- 65 Bhandary B, Marahatta A, Kim H-R & Chae H-J (2012) An involvement of oxidative stress in endoplasmic reticulum stress and its associated diseases. *Int J Mol Sci* **14**, 434–456.
- 66 Cadenas E & Davies KJ (2000) Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* **29**, 222–230.
- 67 Knupp J, Arvan P & Chang A (2018) Increased mitochondrial respiration promotes survival from endoplasmic reticulum stress. *Cell Death Differ.* <https://doi.org/10.1038/s41418-018-0133-4>
- 68 Tu BP & Weissman JS (2004) Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol* **164**, 341–346.
- 69 Malhotra JD & Kaufman RJ (2007) Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid Redox Signal* **9**, 2277–2293.
- 70 Cao SS & Kaufman RJ (2014) Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. *Antioxid Redox Signal* **21**, 396–413.
- 71 Montibeller L & de Belleruche J (2018) Amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD) are characterised by differential activation of ER stress pathways: focus on UPR target genes. *Cell Stress Chaperones*. <https://doi.org/10.1007/s12192-018-0897-y>
- 72 Bertolotti A, Zhang Y, Hendershot LM, Harding HP & Ron D (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat Cell Biol* **2**, 326–332.
- 73 Shen J, Chen X, Hendershot L & Prywes R (2002) ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. *Dev Cell* **3**, 99–111.
- 74 Kopp MC, Nowak PR, Larburu N, Adams CJ & Ali MMU (2018) *In vitro* FRET analysis of IRE1 and BiP association and dissociation upon endoplasmic reticulum stress. *Elife* **7**, e30257.
- 75 Amin-Wetzel N, Saunders RA, Kamphuis MJ, Rato C, Preissler S, Harding HP & Ron D (2017) A J-Protein co-chaperone recruits BiP to monomerize IRE1 and repress the unfolded protein response. *Cell* **171**, 1625–1637.e13.
- 76 Sepulveda D, Rojas-Rivera D, Rodríguez DA, Groenendyk J, Köhler A, Lebeaupin C, Ito S, Urra H, Carreras-Sureda A, Hazari Y *et al.* (2018) Interactome screening identifies the ER luminal chaperone Hsp47 as a regulator of the unfolded protein response transducer IRE1 $\alpha$ . *Mol Cell* **69**, 238–252.e7.
- 77 Ye J, Rawson RB, Komuro R, Chen X, Davé UP, Prywes R, Brown MS & Goldstein JL (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* **6**, 1355–1364.

- 78 Carrara M, Prischi F & Ali MMU (2013) UPR signal activation by luminal sensor domains. *Int J Mol Sci* **14**, 6454–6466.
- 79 Tirasophon W, Welihinda AA & Kaufman RJ (1998) A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev* **12**, 1812–1824.
- 80 Wang XZ, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M & Ron D (1998) Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J* **17**, 5708–5717.
- 81 Iwawaki T, Hosoda A, Okuda T, Kamigori Y, Nomura-Furuwatari C, Kimata Y, Tsuru A & Kohno K (2001) Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. *Nat Cell Biol* **3**, 158–165.
- 82 Bertolotti A, Wang X, Novoa I, Jungreis R, Schlessinger K, Cho JH, West AB & Ron D (2001) Increased sensitivity to dextran sodium sulfate colitis in IRE1 $\beta$ -deficient mice. *J Clin Invest* **107**, 585–593.
- 83 Martino MB, Jones L, Brighton B, Ehre C, Abdulah L, Davis CW, Ron D, O'Neal WK & Ribeiro CMP (2013) The ER stress transducer IRE1 $\beta$  is required for airway epithelial mucin production. *Mucosal Immunol* **6**, 639–654.
- 84 Iwawaki T, Akai R, Yamanaka S & Kohno K (2009) Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. *Proc Natl Acad Sci USA* **106**, 16657–16662.
- 85 Bouchecareilh M, Higa A, Fribourg S, Moenner M & Chevet E (2011) Structure of the Ire1 autophosphorylation complex and implications for the unfolded protein response. *FASEB J* **25**, 3115–3129.
- 86 Sanches M, Duffy NM, Talukdar M, Thevakumaran N, Chiovitti D, Canny MD, Lee K, Kurinov I, Uehling D, Al-awar R *et al.* (2014) Structure and mechanism of action of the hydroxy-aryl-aldehyde class of IRE1 endoribonuclease inhibitors. *Nat Commun* **5**, 4202.
- 87 Prischi F, Nowak PR, Carrara M & Ali MMU (2014) Phosphoregulation of Ire1 RNase splicing activity. *Nat Commun* **5**, 3554.
- 88 Urano F, Wang X-Z, Bertolotti A, Zhang Y, Chung P, Harding HP & Ron D (2000) Coupling of stress in the endoplasmic reticulum to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* **287**, 664–666.
- 89 Bork P & Sander C (1993) A hybrid protein kinase-RNase in an interferon-induced pathway? *FEBS Lett* **334**, 149–152.
- 90 Sidrauski C & Walter P (1997) The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell* **90**, 1031–1039.
- 91 Yoshida H, Matsui T, Yamamoto A, Okada T & Mori K (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107**, 881–891.
- 92 Greer CL, Peebles CL, Gegenheimer P & Abelson J (1983) Mechanism of action of a yeast RNA ligase in tRNA splicing. *Cell* **32**, 537–546.
- 93 Sidrauski C, Cox JS & Walter P (1996) tRNA ligase is required for regulated mRNA splicing in the unfolded protein response. *Cell* **87**, 405–413.
- 94 Schwer B, Sawaya R, Ho CK & Shuman S (2004) Portability and fidelity of RNA-repair systems. *Proc Natl Acad Sci USA* **101**, 2788–2793.
- 95 Tanaka N, Meineke B & Shuman S (2011) RtcB, a novel RNA ligase, can catalyze tRNA splicing and HAC1 mRNA splicing *in vivo*. *J Biol Chem* **286**, 30253–30257.
- 96 Baltz AG, Munschauer M, Schwanhäusser B, Vasile A, Murakawa Y, Schueler M, Youngs N, Penfold-Brown D, Drew K, Milek M *et al.* (2012) The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. *Mol Cell* **46**, 674–690.
- 97 Lu Y, Liang FX & Wang X (2014) A synthetic biology approach identifies the mammalian UPR RNA ligase RtcB. *Mol Cell* **55**, 758–770.
- 98 Liou HC, Boothby MR, Finn PW, Davidon R, Nabavi N, Zeleznik-Le NJ, Ting JP & Glimcher LH (1990) A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. *Science* **247**, 1581–1584.
- 99 Yoshimura T, Fujisawa J & Yoshida M (1990) Multiple cDNA clones encoding nuclear proteins that bind to the tax-dependent enhancer of HTLV-1: all contain a leucine zipper structure and basic amino acid domain. *EMBO J* **9**, 2537–2542.
- 100 Nojima H, Leem Sh, Araki H, Sakai A, Nakashima N, Kanaoka Y & Ono Y (1994) Hac1: a novel yeast bZIP protein binding to the CRE motif is a multicopy suppressor for cdcW mutant of *Schizosaccharomyces pombe*. *Nucleic Acids Res* **22**, 5279–5288.
- 101 Cox JS & Walter P (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* **87**, 391–404.
- 102 Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG & Ron D (2002) IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415**, 92–96.
- 103 Chen CY, Malchus NS, Hehn B, Stelzer W, Avci D, Langosch D & Lemberg MK (2014) Signal peptide peptidase functions in ERAD to cleave the unfolded

- protein response regulator XBP1 $\alpha$ . *EMBO J* **33**, 2492–2506.
- 104 Yanagitani K, Imagawa Y, Iwakoshi T, Hosoda A, Saito M, Kimata Y & Kohno K (2009) Cotranslational Targeting of XBP1 protein to the membrane promotes cytoplasmic splicing of its own mRNA. *Mol Cell* **34**, 191–200.
- 105 Yanagitani K, Kimata Y, Kadokura H & Kohno K (2011) Translational pausing ensures membrane targeting and cytoplasmic splicing of XBP1 $\alpha$  mRNA. *Science* **331**, 586–589.
- 106 Kanda S, Yanagitani K, Yokota Y, Esaki Y & Kohno K (2016) Autonomous translational pausing is required for XBP1 $\alpha$  mRNA recruitment to the ER via the SRP pathway. *Proc Natl Acad Sci* **113**, E5886–E5895.
- 107 Chalmers F, Sweeney B, Cain K & Bulleid NJ (2017) Inhibition of IRE1 $\alpha$ -mediated XBP1 mRNA cleavage by XBP1 reveals a novel regulatory process during the unfolded protein response. *Wellcome Open Res* **2**, 36.
- 108 He Y, Sun S, Sha H, Liu Z, Yang L, Xue Z, Chen H & Qi L (2010) Emerging roles for XBP1, a sUPeR transcription factor. *Gene Expr* **15**, 13–25.
- 109 Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS & Walter P (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* **101**, 249–258.
- 110 Iwakoshi NN, Lee A-H & Glimcher LH (2003) The X-box binding protein-1 transcription factor is required for plasma cell differentiation and the unfolded protein response. *Immunol Rev* **194**, 29–38.
- 111 Sriburi R, Jackowski S, Mori K & Brewer JW (2004) XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. *J Cell Biol* **167**, 35–41.
- 112 Korchak HM (2008) Regulation of hepatic lipogenesis. *Tufts Folia Med* **8**, 134–143.
- 113 So JS, Hur KY, Tarrío M, Ruda V, Frank-Kamenetsky M, Fitzgerald K, Koteliansky V, Lichtman AH, Iwakoshi T, Glimcher LH *et al.* (2012) Silencing of lipid metabolism genes through IRE1 $\alpha$ -mediated mRNA decay lowers plasma lipids in mice. *Cell Metab* **16**, 487–499.
- 114 Zhou Y, Lee J, Reno CM, Sun C, Park SW, Chung J, Lee J, Fisher SJ, White MF, Biddinger SB *et al.* (2011) Regulation of glucose homeostasis through a XBP-1–FoxO1 interaction. *Nat Med* **17**, 356–365.
- 115 Park SW, Herrema H, Salazar M, Cakir I, Cabi S, Basibuyuk Sahin F, Chiu YH, Cantley LC & Ozcan U (2014) BRD7 regulates XBP1s' activity and glucose homeostasis through its interaction with the regulatory subunits of PI3K. *Cell Metab* **20**, 73–84.
- 116 Liu J, Ibi D, Taniguchi K, Lee J, Herrema H, Akosman B, Mucka P, Salazar Hernandez MA, Uyar MF, Park SW *et al.* (2016) Inflammation improves glucose homeostasis through IKK $\beta$ -XBP1s interaction. *Cell* **167**, 1052–1066.e18.
- 117 Lee J, Sun C, Zhou Y, Lee J, Gokalp D, Herrema H, Park SW, Davis RJ & Ozcan U (2011) p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. *Nat Med* **17**, 1251–1260.
- 118 Yingfeng D, Zhao WV, Caroline T, Ningguo G, William HL, Anwarul F, Joyce RJ, Guosheng L, Jin Y, Mark LA *et al.* (2013) The Xbp1s/GalE axis links ER stress to postprandial hepatic metabolism. *J Clin Invest* **123**, 455–468.
- 119 Özcan U, Cao Q, Yilmaz E, Lee A-H, Iwakoshi NN, Özdelen E, Tuncman G, Görgün C, Glimcher Laurie H & Hotamisligil GS (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306**, 457–461.
- 120 Akiyama M, Liew CW, Lu S, Hu J, Martinez R, Hambro B, Kennedy RT & Kulkarni RN (2013) X-box binding protein 1 is essential for insulin regulation of pancreatic  $\alpha$ -cell function. *Diabetes* **62**, 2439–2449.
- 121 Liu Y, Adachi M, Zhao S, Hareyama M, Koong AC, Luo D & Rando TA (2010) Preventing oxidative stress a new role for XBP1. *Cell* **16**, 847–857.
- 122 Tao R, Chen H, Gao C, Xue P, Yang F, Han J-DJ, Zhou B & Chen Y-G (2011) Xbp1-mediated histone H4 deacetylation contributes to DNA double-strand break repair in yeast. *Cell Res* **21**, 1619–1633.
- 123 Wu J & Kaufman RJ (2006) From acute ER stress to physiological roles of the Unfolded Protein Response. *Cell Death Differ* **13**, 374–384.
- 124 Blais A, Tsikitis M, Acosta-Alvear D, Sharan R, Kluger Y & Dynlacht BD (2005) An initial blueprint for myogenic differentiation. *Genes Dev* **19**, 553–569.
- 125 Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, Gravalles EM, Friend D, Grusby MJ, Alt F & Glimcher LH (2001) Plasma cell differentiation requires the transcription factor XBP-1. *Nature* **412**, 300–307.
- 126 Lee A-H, Chu GC, Iwakoshi NN & Glimcher LH (2005) XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. *EMBO J* **24**, 4368–4380.
- 127 Huh WJ, Esen E, Geahlen JH, Bredemeyer AJ, Lee A, Shi G, Konieczny SF, Glimcher LH & Mills JC (2010) XBP1 controls maturation of gastric zymogenic cells by induction of MIST1 and expansion of the rough endoplasmic reticulum. *Gastroenterology* **139**, 2038–2049.
- 128 Sha H, He Y, Chen H, Wang C, Zenno A, Shi H, Yang X & Zhang X (2009) The IRE1 $\alpha$ -XBP1 pathway of the unfolded protein response is required for adipogenesis. *Cell Metab* **9**, 556–564.
- 129 Masaki T, Yoshida M & Noguchi S (1999) Targeted disruption of CRE-Binding factor TREB5 gene leads

- to cellular necrosis in cardiac myocytes at the embryonic stage. *Biochem Biophys Res Commun* **261**, 350–356.
- 130 Reimold AM, Etkin A, Clauss I, Perkins A, Friend DS, Zhang J, Horton HF, Scott A, Orkin SH, Byrne MC *et al.* (2000) An essential role in liver development for transcription factor XBP-1. *Genes Dev* **14**, 152–157.
- 131 Sone M, Zeng X, Larese J & Ryoo HD (2013) A modified UPR stress sensing system reveals a novel tissue distribution of IRE1/XBP1 activity during normal *Drosophila* development. *Cell Stress Chaperones* **18**, 307–319.
- 132 Ono SJ, Liout H, Davidov R, Strominger JL & Glimchert LH (1991) Human X-box-binding protein 1 is required for the transcription of a subset of human class II major histocompatibility genes and forms a heterodimer with c-fos. *Proc Natl Acad Sci USA* **88**, 4309–4312.
- 133 Ding L, Yan J, Zhu J, Zhong H, Lu Q, Wang Z, Huang C & Ye Q (2003) Ligand-independent activation of estrogen receptor  $\alpha$  by XBP-1. *Nucleic Acids Res* **31**, 5266–5274.
- 134 Ravasi T, Suzuki H, Cannistraci CV, Katayama S, Bajic VB, Tan K, Akalin A, Schmeier S, Kanamori-Katayama M, Bertin N *et al.* (2010) An atlas of combinatorial transcriptional regulation in mouse and man. *Cell* **140**, 744–752.
- 135 Reinke AW, Baek J, Ashenberg O & Keating AE (2013) Networks of bZIP protein-protein interactions diversified over a billion years of evolution. *Science* **340**, 730–734.
- 136 Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziaepostolou M, Lim E, Tam WL, Ni M, Chen Y *et al.* (2014) XBP1 promotes triple-negative breast cancer by controlling the HIF1 $\alpha$  pathway. *Nature* **508**, 103–107.
- 137 Hollien J, Lin JH, Li H, Stevens N, Walter P & Weissman JS (2009) Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J Cell Biol* **186**, 323–331.
- 138 Maurel M, Chevet E, Tavernier J & Gerlo S (2014) Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem Sci* **39**, 245–254.
- 139 Lhomond S, Avril T, Dejeans N, Voutetakis K, Doultinos D, McMahon M, Pineau R, Obacz J, Papadodima O, Jouan F *et al.* (2018) Dual IRE1 RNase functions dictate glioblastoma development. *EMBO Mol Med* **10**, e7929. <https://doi.org/10.15252/emmm.201707929>
- 140 Tirasophon W, Lee K, Callaghan B, Welihinda A & Kaufman RJ (2000) The endoribonuclease activity of mammalian IRE1 autoregulates its mRNA and is required for the unfolded protein response. *Genes Dev* **14**, 2725–2736.
- 141 Hollien J & Weissman JS (2006) Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* **313**, 104–107.
- 142 Han D, Lerner AG, Vande WL, Upton J-P, Xu W, Hagen A, Backes BJ, Oakes SA & Papa FR (2009) IRE1 $\alpha$  kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell* **138**, 562–575.
- 143 Kimmig P, Diaz M, Zheng J, Williams CC, Lang A, Aragón T, Li H & Walter P (2012) The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis. *Elife* **2012**, 1–20.
- 144 Mishiba K-0049, Nagashima Y, Suzuki E, Hayashi N, Ogata Y, Shimada Y & Koizumi N (2013) Defects in IRE1 enhance cell death and fail to degrade mRNAs encoding secretory pathway proteins in the Arabidopsis unfolded protein response. *Proc Natl Acad Sci* **110**, 5713–5718.
- 145 Hayashi S, Wakasa Y, Ozawa K & Takaiwa F (2016) Characterization of IRE1 ribonuclease-mediated mRNA decay in plants using transient expression analyses in rice protoplasts. *New Phytol* **210**, 1259–1268.
- 146 Oikawa D, Tokuda M, Hosoda A & Iwawaki T (2010) Identification of a consensus element recognized and cleaved by IRE1 $\alpha$ . *Nucleic Acids Res* **38**, 6265–6273.
- 147 Iqbal J, Dai K, Seimon T, Jungreis R, Oyadomari M, Kuriakose G, Ron D, Tabas I & Hussain MM (2008) IRE1 $\beta$  Inhibits chylomicron production by selectively degrading MTP mRNA. *Cell Metab* **7**, 445–455.
- 148 Imagawa Y, Hosoda A, Sasaka S, Tsuru A & Kohno K (2008) RNase domains determine the functional difference between IRE1 $\alpha$  and IRE1 $\beta$ . *FEBS Lett* **582**, 656–660.
- 149 Shi Y, Vattem KM, Sood R, An J, Liang J, Stramm L & Wek RC (1998) Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. *Mol Cell Biol* **18**, 7499–7509.
- 150 Harding HP, Zhang Y & Ron D (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* **397**, 271–274.
- 151 McQuiston A & Diehl JA (2017) Recent insights into PERK-dependent signaling from the stressed endoplasmic reticulum. *F1000Research* **6**, 1897.
- 152 Lloyd MA, Osborne JC, Safer B, Powell GM & Merrick WC (1980) Characteristics of eukaryotic initiation factor 2 and its subunits. *J Biol Chem* **255**, 1189–1193.
- 153 Ernst H, Duncan RF & Hershey JWB (1987) Cloning and sequencing of complementary DNAs encoding the

- alpha-subunit of translational initiation factor-Eif-2 - characterization of the protein and its messenger RNA. *J Biol Chem* **262**, 1206–1212.
- 154 Adams SL, Safer B & Anderson WFMW (1975) Eukaryotic initiation complex formation. Evidence for two distinct pathways. *J Biol Chem* **250**, 9083–9089.
- 155 Rowlands AG, Panniers R & Henshaw EC (1988) The catalytic mechanism of guanine nucleotide exchange factor action and competitive inhibition by phosphorylated eukaryotic initiation factor 2. *J Biol Chem* **263**, 5526–5533.
- 156 Harding HP, Zhang Y, Bertolotti A, Zeng H & Ron D (2000) Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell* **5**, 897–904.
- 157 Hai T, Liu F, Coukos WJ & Green MR (1989) Transcription factor ATF cDNA clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. *Genes Dev* **3**, 2083–2090.
- 158 Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M & Ron D (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* **6**, 1099–1108.
- 159 Vallejo M, Ron D, Miller CP & Habener JF (1993) C/ATF, a member of the activating transcription factor family of DNA-binding proteins, dimerizes with CAAT/enhancer-binding proteins and directs their binding to cAMP response elements. *Proc Natl Acad Sci USA* **90**, 4679–4683.
- 160 Fawcett TW, Martindale JL, Guyton KZ, Hai T & Holbrook NJ (1999) Complexes containing activating transcription factor (ATF)/cAMP-responsive-element-binding protein (CREB) interact with the CCAAT/enhancer-binding protein (C/EBP)–ATF composite site to regulate Gadd153 expression during the stress response. *Biochem J* **339**, 135–141.
- 161 Han J, Back SH, Hur J, Lin Y, Gildersleeve R, Shan J, Yuan CL, Krokowski D, Wang S, Hatzoglou M *et al.* (2013) ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat Cell Biol* **15**, 481–490.
- 162 Hiramatsu N, Messah C, Han J, LaVail MM, Kaufman RJ & Lin JH (2014) Translational and posttranslational regulation of XIAP by eIF2 $\alpha$  and ATF4 promotes ER stress-induced cell death during the unfolded protein response. *Mol Biol Cell* **25**, 1411–1420.
- 163 Chen YJ, Tan BCM, Cheng YY, Chen JS & Lee SC (2009) Differential regulation of CHOP translation by phosphorylated eIF4E under stress conditions. *Nucleic Acids Res* **38**, 764–777.
- 164 Palam LR, Baird TD & Wek RC (2011) Phosphorylation of eIF2 facilitates ribosomal bypass of an inhibitory upstream ORF to enhance CHOP translation. *J Biol Chem* **286**, 10939–10949.
- 165 Barbosa C, Peixeiro I & Romão L (2013) Gene expression regulation by upstream open reading frames and human disease. *PLoS Genet* **9**, 1–12.
- 166 Fornace AJ, Alamo I & Hollander MC (1988) DNA damage-inducible transcripts in mammalian cells. *Proc Natl Acad Sci USA* **85**, 8800–8804.
- 167 Fornace AJ, Nebert DW, Hollander MC, Luethy JD, Papatianasiou M, Fargnoli J & Holbrook NJ (1989) Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. *Mol Cell Biol* **9**, 4196–4203.
- 168 Lee YY, Cevallos RC & Jan E (2009) An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2 phosphorylation. *J Biol Chem* **284**, 6661–6673.
- 169 Ma Y & Hendershot LM (2003) Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress. *J Biol Chem* **278**, 34864–34873.
- 170 Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP & Ron D (2004) CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev* **18**, 3066–3077.
- 171 Connor JH, Weiser DC, Li S, John M, Li SHI & Hallenbeck JM (2001) Growth arrest and DNA damage-inducible protein GADD34 assembles a novel signaling complex containing Protein Phosphatase 1 and inhibitor 1. *Mol Cell Biol* **21**, 6841–6850.
- 172 Novoa I, Zeng H, Harding HP & Ron D (2001) Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2 $\alpha$ . *J Cell Biol* **153**, 1011–1022.
- 173 Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R *et al.* (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* **11**, 619–633.
- 174 Quirós PM, Prado MA, Zamboni N, D'Amico D, Williams RW, Finley D, Gygi SP & Auwerx J (2017) Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. *J Cell Biol* **216**, 2027–2045.
- 175 Rajesh K, Krishnamoorthy J, Kazimierczak U, Tenkerian C, Papadakis AI, Wang S, Huang S & Koromilas AE (2015) Phosphorylation of the translation initiation factor eIF2 $\alpha$  at serine 51 determines the cell fate decisions of Akt in response to oxidative stress. *Cell Death Dis* **6**, e1591.
- 176 Lee IC, Ho XY, George SE, Goh CW, Sundaram JR, Pang KKL, Luo W, Yusoff P, Sze NSK & Shenolikar S (2017) Oxidative stress promotes SIRT1 recruitment to the GADD34/PP1 $\alpha$  complex to activate its deacetylase function. *Cell Death Differ* **25**, 255–267.

- 177 Chen D, Fan Z, Rauh M, Buchfelder M, Eyupoglu IY & Savaskan N (2017) ATF4 promotes angiogenesis and neuronal cell death and confers ferroptosis in a xCT-dependent manner. *Oncogene* **36**, 5593–5608.
- 178 Wu Z, Li M, Zheng W, Hu Q, Cheng Z & Guo F (2017) Silencing of both ATF4 and PERK inhibits cell cycle progression and promotes the apoptosis of differentiating chondrocytes. *Int J Mol Med* **40**, 101–111.
- 179 Ishizawa J, Kojima K, Chachad D, Ruvolo P, Ruvolo V, Jacamo RO, Borthakur G, Mu H, Zeng Z, Tabe Y *et al.* (2016) ATF4 induction through an atypical integrated stress response to ONC201 triggers p53-independent apoptosis in hematological malignancies. *Sci Signal* **9**, ra17.
- 180 Iurlaro R, Püschel F, León-Annicchiarico CL, O'Connor H, Martin SJ, Palou-Gramón D, Lucendo E & Muñoz-Pinedo C (2017) Glucose deprivation induces ATF4-mediated apoptosis through TRAIL death receptors. *Mol Cell Biol* **37**, e00479–16. <https://doi.org/10.1128/MCB.00479-16>
- 181 Zhu C, Johansen FE & Prywes R (1997) Interaction of ATF6 and serum response factor. *Mol Cell Biol* **17**, 4957–4966.
- 182 Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H, Harada A & Mori K (2007) Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev Cell* **13**, 365–376.
- 183 Asada R, Kanemoto S, Kondo S, Saito A & Imaizumi K (2011) The signalling from endoplasmic reticulum-resident bZIP transcription factors involved in diverse cellular physiology. *J Biochem* **149**, 507–518.
- 184 McMahan M, Samali A & Chevet E (2017) Regulation of the unfolded protein response by noncoding RNA. *Am J Physiol Cell Physiol* **313**, C243–C254.
- 185 Osowski CM & Urano F (2011) Measuring ER stress and the unfolded protein response using mammalian tissue culture system. *Methods Enzymol* **490**, 71–92.
- 186 Yoshida H, Matsui T, Hosokawa N, Kaufman RJ, Nagata K & Mori K (2003) A time-dependent phase shift in the mammalian unfolded protein response. *Dev Cell* **4**, 265–271.
- 187 Sano R & Reed JC (2013) ER stress-induced cell death mechanisms. *Biochim Biophys Acta* **1833**, 3460–3470.
- 188 Bartoszewski S, Kochan K, Madanecki P, Piotrowski A, Ochocka R, Collawn JF & Bartoszewski R (2013) Regulation of the unfolded protein response by microRNAs. *Cell Mol Biol Lett* **18**, 555–578.
- 189 Wortel IMN, van der Meer LT, Kilberg MS & van Leeuwen FN (2017) Surviving stress: modulation of ATF4-mediated stress responses in normal and malignant cells. *Trends Endocrinol Metab* **28**, 794–806.
- 190 Gorman AM, Healy SJ, Jager R & Samali A (2012) Stress management at the ER: regulators of ER stress-induced apoptosis. *Pharmacol Ther* **134**, 306–316.
- 191 Ron D & Walter P (2007) Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* **8**, 519–529.
- 192 Hoyer-Hansen M & Jaattela M (2007) Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ* **14**, 1576–1582.
- 193 Cheng X, Liu H, Jiang CC, Fang L, Chen C, Zhang XD & Jiang ZW (2014) Connecting endoplasmic reticulum stress to autophagy through IRE1/JNK/beclin-1 in breast cancer cells. *Int J Mol Med* **34**, 772–781.
- 194 Giorgi C, Missiroli S, Patergnani S, Duszynski J, Wieckowski MR & Pinton P (2015) Mitochondria-associated membranes: composition, molecular mechanisms, and physiopathological implications. *Antioxid Redox Signal* **22**, 995–1019.
- 195 Santel A & Fuller MT (2001) Control of mitochondrial morphology by a human mitofusin. *J Cell Sci* **114**, 867–874.
- 196 Muñoz JP, Ivanova S, Sánchez-Wandelmer J, Martínez-Cristóbal P, Noguera E, Sancho A, Díaz-Ramos A, Hernández-Alvarez MI, Sebastián D, Mauvezin C *et al.* (2014) Erratum: Mfn2 modulates the UPR and mitochondrial function via repression of PERK (EMBO Journal 32 (2348-2361)) <https://doi.org/10.1038/emboj.2013.168>. *EMBO J* **33**, 171.
- 197 Verfaillie T, Rubio N, Garg AD, Bultynck G, Rizzuto R, Decuypere JP, Piette J, Linehan C, Gupta S, Samali A *et al.* (2012) PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ* **19**, 1880–1891.
- 198 Mori T, Hayashi T, Hayashi E & Su TP (2013) Sigma-1 receptor chaperone at the er-mitochondrion interface mediates the mitochondrion-er-nucleus signaling for cellular survival. *PLoS ONE* **8**, e76941.
- 199 Lisbona F, Rojas-Rivera D, Thielen P, Zamorano S, Todd D, Martinon F, Glavic A, Kress C, Lin JH, Walter P *et al.* (2009) BAX inhibitor-1 is a negative regulator of the ER stress sensor IRE1alpha. *Mol Cell* **33**, 679–691.
- 200 Vannuel K, Renard P, Raes M & Arnould T (2013) Functional and morphological impact of ER stress on mitochondria. *J Cell Physiol* **228**, 1802–1818.
- 201 Bravo R, Vicencio JM, Parra V, Troncoso R, Munoz JP, Bui M, Quiroga C, Rodriguez AE, Verdejo HE, Ferreira J *et al.* (2011) Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. *J Cell Sci* **124**, 2511.
- 202 Cullinan SB & Diehl JA (2004) PERK-dependent activation of Nrf2 contributes to redox homeostasis

- and cell survival following endoplasmic reticulum stress. *J Biol Chem* **279**, 20108–20117.
- 203 Wang C, Li H, Meng Q, Du Y, Xiao F, Zhang Q, Yu J, Li K, Chen S, Huang Z *et al.* (2014) ATF4 deficiency protects hepatocytes from oxidative stress via inhibiting CYP2E1 expression. *J Cell Mol Med* **18**, 80–90.
- 204 Li G, Mongillo M, Chin KT, Harding H, Ron D, Marks AR & Tabas I (2009) Role of ERO1- $\alpha$ -mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. *J Cell Biol* **186**, 783–792.
- 205 Eletto D, Chevet E, Argon Y & Appenzeller-Herzog C (2014) Redox controls UPR to control redox. *J Cell Sci* **127**, 3649–3658.
- 206 Appenzeller-Herzog C & Hall MN (2012) Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling. *Trends Cell Biol* **22**, 274–282.
- 207 Patricia F, Julien B, Justine L, Patrick M, Joëlle F, Cécile V, Thomas W, Serge M, Colette R, Jean-Yves S *et al.* (2017) mTOR inhibitors activate PERK signaling and favor viability of gastrointestinal neuroendocrine cell lines. *Oncotarget* **8**, 20974–20987.
- 208 Tenkerian C, Krishnamoorthy J, Mounir Z, Kazimierczak U, Khoutorsky A, Staschke KA, Kristof AS, Wang S, Hatzoglou M & Koromilas AE (2015) mTORC2 balances AKT activation and eIF2 $\alpha$  serine 51 phosphorylation to promote survival under stress. *Mol Cancer Res* **13**, 1377–1388.
- 209 Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA *et al.* (2003) The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol* **5**, 781–792.
- 210 Fu S, Yang L, Li P, Hofmann O, Dicker L, Hide W, Lin X, Watkins SM, Ivanov AR & Hotamisligil GS (2011) Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature* **473**, 528–531.
- 211 Kaplowitz N, Than TA, Ph D, Shinohara M & Ji C (2007) Endoplasmic reticulum stress and liver injury. *Semin Liver Dis* **27**, 367–377.
- 212 Oyadomari S, Harding HP, Zhang Y, Oyadomari M & Ron D (2008) De-phosphorylation of translation initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) enhances glucose tolerance and attenuates hepato-steatosis in mice. *Cell Metab* **7**, 520–532.
- 213 Li H, Meng Q, Xiao F, Chen S, Du Y, Yu J, Wang C & Guo F (2011) ATF4 deficiency protects mice from high-carbohydrate-diet-induced liver steatosis. *Biochem J* **438**, 283–289.
- 214 Xiao G, Zhang T, Yu S, Lee S, Calabuig-Navarro V, Yamauchi J, Ringquist S & Dong HH (2013) ATF4 protein deficiency protects against high fructose-induced hypertriglyceridemia in mice. *J Biol Chem* **288**, 25350–25361.
- 215 Wang S, Chen Z, Lam V, Han J, Hassler J, Finck BN, Davidson NO & Kaufman RJ (2012) IRE1 $\alpha$ -XBP1s induces PDI expression to increase MTP activity for hepatic VLDL assembly and lipid homeostasis. *Cell Metab* **16**, 473–486.
- 216 Lee AH, Scapa EF, Cohen DE & Glimcher LH (2008) Regulation of hepatic lipogenesis by the transcription factor XBP1. *Science (80-)* **320**, 1492–1496.
- 217 Yi H, Gu C, Li M, Zhang Z, Li Q, Feng J, Zhou J & Du J (2017) PERK/eIF2 $\alpha$  contributes to changes of insulin signaling in HepG2 cell induced by intermittent hypoxia. *Life Sci* **181**, 17–22.
- 218 Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M *et al.* (2007) Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* **56**, 901–911.
- 219 Kim B, Kim MS & Hyun CK (2017) Syringin attenuates insulin resistance via adiponectin-mediated suppression of low-grade chronic inflammation and ER stress in high-fat diet-fed mice. *Biochem Biophys Res Commun* **488**, 40–45.
- 220 Lipson KL, Fonseca SG, Ishigaki S, Nguyen LX, Foss E, Bortell R, Rossini AA & Urano F (2006) Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. *Cell Metab* **4**, 245–254.
- 221 Lee A-H, Heidtman K, Hotamisligil GS & Glimcher LH (2011) Dual and opposing roles of the unfolded protein response regulated by IRE1 $\alpha$  and XBP1 in proinsulin processing and insulin secretion. *Proc Natl Acad Sci* **108**, 8885–8890.
- 222 Seo H-Y, Kim YD, Lee K-M, Min A-K, Kim M-K, Kim H-S, Won K-C, Park J-Y, Lee K-U, Choi H-S *et al.* (2008) Endoplasmic reticulum stress-induced activation of activating transcription factor 6 decreases insulin gene expression via up-regulation of orphan nuclear receptor small heterodimer partner. *Endocrinology* **149**, 3832–3841.
- 223 Shao M, Shan B, Liu Y, Deng Y, Yan C, Wu Y, Mao T, Qiu Y, Zhou Y, Jiang S *et al.* (2014) Hepatic IRE1 $\alpha$  regulates fasting-induced metabolic adaptive programs through the XBP1s–PPAR $\alpha$  axis signalling. *Nat Commun* **5**, 3528.
- 224 Kim H, Tu HC, Ren D, Takeuchi O, Jeffers JR, Zambetti GP, Hsieh JJ & Cheng EH (2009) Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. *Mol Cell* **36**, 487–499.
- 225 Gomez-Bougie P, Halliez M, Moreau P, Pellat-Deceunynck C & Amiot M (2016) Repression of Mcl-1 and disruption of the Mcl-1/Bak interaction in

- myeloma cells couple ER stress to mitochondrial apoptosis. *Cancer Lett* **383**, 204–211.
- 226 Li J, Lee B & Lee AS (2006) Endoplasmic reticulum stress-induced apoptosis: multiple pathways and activation of p53-up-regulated modulator of apoptosis (PUMA) and NOXA by p53. *J Biol Chem* **281**, 7260–7270.
- 227 Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tanii I, Yoshinaga K *et al.* (2006) Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* **26**, 9220–9231.
- 228 Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, Baehrecke EH & Lenardo MJ (2004) Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. *Science (80-.)* **304**, 1500–1502.
- 229 Kim I, Shu CW, Xu W, Shiao CW, Grant D, Vasile S, Cosford ND & Reed JC (2009) Chemical biology investigation of cell death pathways activated by endoplasmic reticulum stress reveals cytoprotective modulators of ASK1. *J Biol Chem* **284**, 1593–1603.
- 230 Deng X, Xiao L, Lang W, Gao F, Ruvolo P & May WS (2001) Novel Role for JNK as a Stress-activated Bcl2 Kinase. *J Biol Chem* **276**, 23681–23688.
- 231 Puthalakath H, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N *et al.* (2007) ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* **129**, 1337–1349.
- 232 Yamaguchi H & Wang H-G (2004) CHOP is involved in endoplasmic reticulum stress-induced apoptosis by enhancing DR5 expression in human carcinoma cells. *J Biol Chem* **279**, 45495–45502.
- 233 Maytin EV, Ubeda M, Lin JC & Habener JF (2001) Stress-Inducible Transcription Factor CHOP/gadd153 Induces Apoptosis in Mammalian Cells via p38 Kinase-Dependent and -Independent Mechanisms. *Exp Cell Res* **267**, 193–204.
- 234 Wei S-G, Yu Y, Weiss RM & Felder RB (2016) Endoplasmic reticulum stress increases brain MAPK signaling, inflammation and renin-angiotensin system activity and sympathetic nerve activity in heart failure. *Am J Physiol Heart Circ Physiol* **311**, H871–H880.
- 235 Nolan K, Walter F, Tuffy LP, Poeschel S, Gallagher R, Haunsberger S, Bray I, Stallings RL, Concannon CG & Prehn JH (2016) Endoplasmic reticulum stress-mediated upregulation of miR-29a enhances sensitivity to neuronal apoptosis. *Eur J Neurosci* **43**, 640–652.
- 236 Wu Y, Li X, Jia J, Zhang Y, Li J, Zhu Z, Wang H, Tang J & Hu J (2018) Transmembrane E3 ligase RNF183 mediates ER stress-induced apoptosis by degrading Bcl-xL. *Proc Natl Acad Sci USA* **115**, E2762–E2771.
- 237 Galluzzi L, Buqué A, Kepp O, Zitvogel L & Kroemer G (2016) Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol* **17**, 97.
- 238 Garg AD & Agostinis P (2017) Cell death and immunity in cancer: from danger signals to mimicry of pathogen defense responses. *Immunol Rev* **280**, 126–148.
- 239 Fan H, Tang HB, Kang J, Shan L, Song H, Zhu K, Wang J, Ju G & Wang YZ (2015) Involvement of endoplasmic reticulum stress in the necroptosis of microglia/macrophages after spinal cord injury. *Neuroscience* **311**, 362–373.
- 240 Linkermann A & Green DR (2014) Necroptosis. *N Engl J Med* **370**, 455–465.
- 241 Saveljeva S, Mc Laughlin SL, Vandenabeele P, Samali A & Bertrand MJ (2015) Endoplasmic reticulum stress induces ligand-independent TNFR1-mediated necroptosis in L929 cells. *Cell Death Dis* **6**, e1587.
- 242 Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD & Levine B (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* **122**, 927–939.
- 243 Gandin V, Pellei M, Tisato F, Porchia M, Santini C & Marzano C (2012) A novel copper complex induces paraptosis in colon cancer cells via the activation of ER stress signalling. *J Cell Mol Med* **16**, 142–151.
- 244 Fan J, Long H, Li Y, Liu Y, Zhou W, Li Q, Yin G, Zhang N & Cai W (2013) Edaravone protects against glutamate-induced PERK/EIF2 $\alpha$ /ATF4 integrated stress response and activation of caspase-12. *Brain Res* **1519**, 1–8.
- 245 Akamatsu K, Shibata M-A, Ito Y, Sohma Y, Azuma H & Otsuki Y (2009) Riluzole induces apoptotic cell death in human prostate cancer cells via endoplasmic reticulum stress. *Anticancer Res* **29**, 2195–2204.
- 246 Cao J, Dai DL, Yao L, Yu HH, Ning B, Zhang Q, Chen J, Cheng WH, Shen W & Yang ZX (2012) Saturated fatty acid induction of endoplasmic reticulum stress and apoptosis in human liver cells via the PERK/ATF4/CHOP signaling pathway. *Mol Cell Biochem* **364**, 115–129.
- 247 Du S, Zhou J, Jia Y & Huang K (2010) SelK is a novel ER stress-regulated protein and protects HepG2 cells from ER stress agent-induced apoptosis. *Arch Biochem Biophys* **502**, 137–143.
- 248 Li S, Zhao F, Cheng S, Wang X & Hao Y (2013) Uric acid-induced endoplasmic reticulum stress triggers phenotypic change in rat glomerular mesangial cells. *Nephrology* **18**, 682–689.
- 249 Apostolou A, Shen Y, Liang Y, Luo J & Fang S (2008) Armet, a UPR-upregulated protein, inhibits cell proliferation and ER stress-induced cell death. *Exp Cell Res* **314**, 2454–2467.



- 250 Morishima N, Nakanishi K, Takenouchi H, Shibata T & Yasuhiko Y (2002) An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12. *J Biol Chem* **277**, 34287–34294.
- 251 Saito A, Ochiai K, Kondo S, Tsumagari K, Murakami T, Cavener DR & Imaizumi K (2011) Endoplasmic reticulum stress response mediated by the PERK-eIF2(α)-ATF4 pathway is involved in osteoblast differentiation induced by BMP2. *J Biol Chem* **286**, 4809–4818.
- 252 Chang YJ, Chen WY, Huang CY, Liu HH & Wei PL (2015) Glucose-regulated protein 78 (GRP78) regulates colon cancer metastasis through EMT biomarkers and the NRF-2/HO-1 pathway. *Tumor Biol* **36**, 1859–1869.
- 253 Tanjore H, Cheng D-S, Degryse AL, Zoz DF, Abdolrasulnia R, Lawson WE & Blackwell TS (2011) Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. *J Biol Chem* **286**, 30972–30980.
- 254 Mo XT, Zhou WC, Cui WH, Li DL, Li LC, Xu L, Zhao P & Gao J (2015) Inositol-requiring protein 1 - X-box-binding protein 1 pathway promotes epithelial-mesenchymal transition via mediating snail expression in pulmonary fibrosis. *Int J Biochem Cell Biol* **65**, 230–238.
- 255 Minchenko DO, Kharkova AP, Halkin OV, Karbovskiy LL & Minchenko OH (2016) Effect of hypoxia on the expression of genes encoding insulin-like growth factors and some related proteins in u87 glioma cells without IRE1 function. *Endocr Regul* **50**, 43–54.
- 256 Li H, Chen X, Gao Y, Wu J, Zeng F & Song F (2015) XBP1 induces snail expression to promote epithelial- to-mesenchymal transition and invasion of breast cancer cells. *Cell Signal* **27**, 82–89.
- 257 Jin C, Jin Z, Chen N-Z, Lu M, Liu C-B, Hu W-L & Zheng C-G (2016) Activation of IRE1α-XBP1 pathway induces cell proliferation and invasion in colorectal carcinoma. *Biochem Biophys Res Commun* **470**, 75–81.
- 258 Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JHL, Proia TA, Jin DX, Reinhardt F, Ploegh HL, Wang Q *et al.* (2014) Epithelial-to-mesenchymal transition activates PERK-eIF2α and sensitizes cells to endoplasmic reticulum stress. *Cancer Discov* **4**, 702–715.
- 259 Moon SY, Kim HS, Nho KW, Jang YJ & Lee SK (2014) Endoplasmic reticulum stress induces epithelial-mesenchymal transition through autophagy via activation of c-Src kinase. *Nephron Exp Nephrol* **126**, 127–140.
- 260 Greenwood M, Bordieri L, Greenwood MP, Rosso Melo M, Colombari DS, Colombari E, Paton JFR & Murphy D (2014) Transcription factor CREB3L1 regulates vasopressin gene expression in the rat hypothalamus. *J Neurosci* **34**, 3810–3820.
- 261 Azuma Y, Hagiwara D, Lu W, Morishita Y, Suga H, Goto M, Banno R, Sugimura Y, Oyadomari S, Mori K *et al.* (2014) Activating transcription factor 6α is required for the vasopressin neuron system to maintain water balance under dehydration in male mice. *Endocrinology* **155**, 4905–4914.
- 262 Marwarha G, Claycombe K, Schommer J, Collins D & Ghribi O (2016) Palmitate-induced endoplasmic reticulum stress and subsequent C/EBPα homologous protein activation attenuates leptin and Insulin-like growth factor 1 expression in the brain. *Cell Signal* **28**, 1789–1805.
- 263 Marwarha G, Dasari B & Ghribi O (2012) Endoplasmic reticulum stress-induced CHOP activation mediates the down-regulation of leptin in human neuroblastoma SH-SY5Y cells treated with the oysterol 27-hydroxycholesterol. *Cell Signal* **24**, 484–492.
- 264 Martínez-Sánchez N, Seoane-Collazo P, Contreras C, Varela L, Villarroja J, Rial-Pensado E, Buqué X, Aurrekoetxea I, Delgado TC, Vázquez-Martínez R *et al.* (2017) Hypothalamic AMPK-ER Stress-JNK1 axis mediates the central actions of thyroid hormones on energy balance. *Cell Metab* **26**, 212–229.e12.
- 265 Zughair SM, Stauffer BB & McCarty NA (2014) Inflammation and ER stress downregulate BDH2 expression and dysregulate intracellular iron in macrophages. *J Immunol Res* **2014**, 140728.
- 266 Mori K (2009) Signalling pathways in the unfolded protein response: development from yeast to mammals. *J Biochem* **146**, 743–750.
- 267 Michalak M & Gye MC (2015) Endoplasmic reticulum stress in periimplantation embryos. *Clin Exp Reprod Med* **42**, 1–7.
- 268 Li J, Chen Z, Gao LY, Colorni A, Ucko M, Fang S & Du SJ (2015) A transgenic zebrafish model for monitoring xbp1 splicing and endoplasmic reticulum stress *in vivo*. *Mech Dev* **137**, 33–44.
- 269 Ishikawa T, Kashima M, Nagano AJ, Ishikawa-Fujiwara T, Kamei Y, Todo T & Mori K (2017) Unfolded protein response transducer IRE1-mediated signaling independent of XBP1 mRNA splicing is not required for growth and development of medaka fish. *Elife* **6**, 1–29.
- 270 Richardson CE, Kinkel S & Kim DH (2011) Physiological IRE-1-XBP-1 and PEK-1 signaling in *Caenorhabditis elegans* larval development and immunity. *PLoS Genet* **7**, 1–10.
- 271 Shi W, Xu G, Wang C, Sperber SM, Chen Y, Zhou Q, Deng Y & Zhao H (2015) Heat shock 70-kDa protein 5 (Hspa5) is essential for pronephros

- formation by mediating retinoic acid signaling. *J Biol Chem* **290**, 577–589.
- 272 Luo S, Mao C, Lee B & Lee AS (2006) GRP78/BiP Is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development. *Mol Cell Biol* **26**, 5688–5697.
- 273 Zhang X, Szabo E, Michalak M & Opas M (2007) Endoplasmic reticulum stress during the embryonic development of the central nervous system in the mouse. *Int J Dev Neurosci* **25**, 455–463.
- 274 Kratochvílová K, Moráň L, Paďourová S, Stejskal S, Tesařová L, Šimara P, Hampl A, Koutná I & Vaňhara P (2016) The role of the endoplasmic reticulum stress in stemness, pluripotency and development. *Eur J Cell Biol* **95**, 115–123.
- 275 Hao L, Vassena R, Wu G, Han Z, Cheng Y, Latham KE & Sapienza C (2009) The unfolded protein response contributes to preimplantation mouse embryo death in the DDK syndrome. *Biol Reprod* **80**, 944–953.
- 276 Yang Y, Cheung HH, Tu J, Miu KK & Chan WY (2016) New insights into the unfolded protein response in stem cells. *Oncotarget* **7**, 54010–54027.
- 277 Latham KE (2015) Endoplasmic reticulum stress signaling in mammalian oocytes and embryos: life in balance. *Int Rev Cell Mol Biol* **316**, 227–265.
- 278 Gao Y, Sartori DJ, Li C, Yu Q-C, Kushner JA, Simon MC & Diehl JA (2012) PERK is required in the adult pancreas and is essential for maintenance of glucose homeostasis. *Mol Cell Biol* **32**, 5129–5139.
- 279 Bettigole SE, Lis R, Adoro S, Lee AH, Spencer LA & Weller PFGL (2015) The transcription factor XBP1 is selectively required for eosinophil differentiation. *Nat Immunol* **16**, 829–837.
- 280 Leung A, Gregory NS, Allen L-AH & Sluka KA (2017) Regular physical activity prevents chronic pain by altering muscle macrophage phenotype and increasing IL-10 in mice. *Pain* **157**, 70–79.
- 281 Todd DJ, McHeyzer-Williams LJ, Kowal C, Lee AH, Volpe BT, Diamond B, McHeyzer-Williams MG & Glimcher LH (2009) XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development. *J Exp Med* **206**, 2151–2159.
- 282 van Galen P, Kreso A, Mbong N, Kent DG, Fitzmaurice T, Chambers JE, Xie S, Laurenti E, Hermans K, Eppert K *et al.* (2014) The unfolded protein response governs integrity of the haematopoietic stem-cell pool during stress. *Nature* **510**, 268–272.
- 283 Matsuzaki S, Hiratsuka T, Taniguchi M, Shingaki K, Kubo T, Kiya K, Fujiwara T, Kanazawa S, Kanematsu R, Maeda T *et al.* (2015) Physiological ER stress mediates the differentiation of fibroblasts. *PLoS ONE* **10**, 1–11.
- 284 Arendsdorf AM, Diedrichs D & Rutkowski DT (2013) Regulation of the transcriptome by ER stress: non-canonical mechanisms and physiological consequences. *Front Genet* **4**, 1–16.
- 285 Murakami T, Saito A, Hino S, Kondo S, Kanemoto S, Chihara K, Sekiya H, Tsumagari K, Ochiai K, Yoshinaga K *et al.* (2009) Signalling mediated by the endoplasmic reticulum stress transducer OASIS is involved in bone formation. *Nat Cell Biol* **11**, 1205–1211.
- 286 Hamamura K & Yokota H (2007) Stress to endoplasmic reticulum of mouse osteoblasts induces apoptosis and transcriptional activation for bone remodeling. *FEBS Lett* **581**, 1769–1774.
- 287 Cui M, Kanemoto S, Cui X, Kaneko M, Asada R, Matsuhisa K, Tanimoto K, Yoshimoto Y, Shukunami C & Imaizumi K (2015) OASIS modulates hypoxia pathway activity to regulate bone angiogenesis. *Sci Rep* **5**, 16455.
- 288 Kondo S, Murakami T, Tatsumi K, Ogata M, Kanemoto S, Otori K, Iseki K, Wanaka A & Imaizumi K (2005) OASIS, a CREB/ATF-family member, modulates UPR signalling in astrocytes. *Nat Cell Biol* **7**, 186–194.
- 289 Asada R, Saito A, Kawasaki N, Kanemoto S, Iwamoto H, Oki M, Miyagi H, Izumi S & Imaizumi K (2012) The endoplasmic reticulum stress transducer OASIS is involved in the terminal differentiation of goblet cells in the large intestine. *J Biol Chem* **287**, 8144–8153.
- 290 Saito A (2014) Physiological functions of endoplasmic reticulum stress transducer OASIS in central nervous system. *Anat Sci Int* **89**, 11–20.
- 291 van der Harg JM, van Heest JC, Bangel FN, Patiwaal S, van Weering JRT & Scheper W (2017) The UPR reduces glucose metabolism via IRE1 signaling. *Biochim Biophys Acta* **1864**, 655–665.
- 292 Topisirovic I & Sonenberg N (2011) mRNA translation and energy metabolism in cancer: the role of the MAPK and mTORC1 Pathways. *Cold Spring Harb Symp Quant Biol* **76**, 355–367.
- 293 Lowe CE, Dennis RJ, Obi U, O'Rahilly S & Rochford JJ (2012) Investigating the involvement of the ATF6 $\alpha$  pathway of the unfolded protein response in adipogenesis. *Int J Obes (Lond)* **36**, 1248–1251.
- 294 Lipson KL, Ghosh R & Urano F (2008) The role of IRE1 $\alpha$  in the degradation of insulin mRNA in pancreatic  $\beta$ -cells. *PLoS ONE* **3**, 1–7.
- 295 Zhang N, Yang X, Yuan F, Zhang L, Wang Y, Wang L, Mao Z, Luo J, Zhang H, Zhu WG *et al.* (2018) Increased amino acid uptake supports autophagy-deficient cell survival upon glutamine deprivation. *Cell Rep* **10**, 3006–3020.

- 296 Rubio-Patiño C, Bossowski JP, De Donatis GM, Mondragón L, Villa E, Aira LE, Chiche J, Mhaidly R, Lebeauin C, Marchetti S *et al.* (2018) Low-protein diet induces IRE1 $\alpha$ -dependent anticancer immunosurveillance. *Cell Metab* **27**, 828–842.
- 297 Xue Z, He Y, Ye K, Gu Z, Mao Y & Qi L (2011) A conserved structural determinant located at the interdomain region of mammalian inositol-requiring enzyme 1 $\alpha$ . *J Biol Chem* **286**, 30859–30866.
- 298 Waller DD, Jansen G, Golizeh M, Martel-Lorion C, Dejgaard K, Shiao TC, Mancuso J, Tsantrizos YS, Roy R, Sebag M *et al.* (2016) A covalent cysteine-targeting kinase inhibitor of Ire1 permits allosteric control of endoribonuclease activity. *ChemBioChem* **17**, 843–851.
- 299 Wiseman RL, Zhang Y, Lee KPK, Harding HP, Haynes CM, Price J, Sicheri F & Ron D (2010) Flavonol activation defines an unanticipated ligand-binding site in the kinase-RNase domain of IRE1. *Mol Cell* **38**, 291–304.
- 300 Rong J, Pass I, Diaz PW, Ngo TA, Sauer M, Magnuson G, Zeng F-Y, Hassig CA, Jackson MR, Cosford NDP *et al.* (2015) Cell-based high-throughput luciferase reporter gene assays for identifying and profiling chemical modulators of endoplasmic reticulum signaling protein, IRE1. *J Biomol Screen* **20**, 1232–1245.
- 301 Feldman HC, Tong M, Wang L, Meza-Acevedo R, Gobillot TA, Lebedev I, Gliedt MJ, Hari SB, Mitra AK, Backes BJ *et al.* (2016) Structural and functional analysis of the allosteric inhibition of IRE1 $\alpha$  with ATP-competitive ligands. *ACS Chem Biol* **11**, 2195–2205.
- 302 Harrington PE, Biswas K, Malwitz D, Tasker AS, Mohr C, Andrews KL, Dellamaggiore K, Kendall R, Beckmann H, Jaeckel P *et al.* (2015) Unfolded protein response in cancer: IRE1 $\alpha$  inhibition by selective kinase ligands does not impair tumor cell viability. *ACS Med Chem Lett* **6**, 68–72.
- 303 Wang L, Perera BGK, Hari SB, Bhatarai B, Backes BJ, Seeliger MA, Schürer SC, Oakes SA, Papa FR & Maly DJ (2012) Divergent allosteric control of the IRE1 $\alpha$  endoribonuclease using kinase inhibitors. *Nat Chem Biol* **8**, 982–989.
- 304 Jha BK, Polyakova I, Kessler P, Dong B, Dickerman B, Sen GC & Silverman RH (2011) Inhibition of RNase L and RNA-dependent protein kinase (PKR) by sunitinib impairs antiviral innate immunity. *J Biol Chem* **286**, 26319–26326.
- 305 Volkmann K, Lucas JL, Vuga D, Wang X, Brumm D, Stiles C, Kriebel D, Der-Sarkissian A, Krishnan K, Schweitzer C *et al.* (2011) Potent and selective inhibitors of the inositol-requiring enzyme 1 endoribonuclease. *J Biol Chem* **286**, 12743–12755.
- 306 Cross BC, Bond PJ, Sadowski PG, Jha BK, Zak J, Goodman JM, Silverman RH, Neubert TA, Baxendale IR, Ron D *et al.* (2012) The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. *Proc Natl Acad Sci USA* **109**, E869–E878.
- 307 Mimura N, Fulciniti M, Gorgun G, Tai Y-T, Cirstea D, Santo L, Hu Y, Fabre C, Minami J, Ohguchi H *et al.* (2012) Blockade of XBP1 splicing by inhibition of IRE1 is a promising therapeutic option in multiple myeloma. *Blood* **119**, 5772–5781.
- 308 Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, Solow-Cordero DE, Bouley DM, Offner F, Niwa M *et al.* (2011) Identification of an Ire1 $\alpha$  endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood* **117**, 1311–1314.
- 309 Ri M, Tashiro E, Oikawa D, Shinjo S, Tokuda M, Yokouchi Y, Narita T, Masaki A, Ito A, Ding J *et al.* (2012) Identification of Toyocamycin, an agent cytotoxic for multiple myeloma cells, as a potent inhibitor of ER stress-induced XBP1 mRNA splicing. *Blood Cancer J* **2**, e79.
- 310 Tomasio SM, Harding HP, Ron D, Cross BCS & Bond PJ (2013) Selective inhibition of the unfolded protein response: targeting catalytic sites for Schiff base modification. *Mol Biosyst* **9**, 2408–2416.
- 311 Jiang D, Lynch C, Medeiros BC, Liedtke M, Bam R, Tam AB, Yang Z, Alagappan M, Abidi P, Le Q-T *et al.* (2016) Identification of Doxorubicin as an Inhibitor of the IRE1 $\alpha$ -XBP1 Axis of the Unfolded Protein Response. *Sci Rep* **6**, 33353.
- 312 Park BK, Boobis A, Clarke S, Goldring CEP, Jones D, Kenna JG, Lambert C, Lavery HG, Naisbitt DJ, Nelson S *et al.* (2011) Managing the challenge of chemically reactive metabolites in drug development. *Nat Rev Drug Discov* **10**, 292–306.
- 313 Wilson AJ (2009) Inhibition of protein–protein interactions using designed molecules. *Chem Soc Rev* **38**, 3289.
- 314 Atkins C, Liu Q, Minthorn E, Zhang S-Y, Figueroa DJ, Moss K, Stanley TB, Sanders B, Goetz A, Gaul N *et al.* (2013) Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res* **73**, 1993–2002.
- 315 Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, Grant SW, Li WH, Heerding DA, Minthorn E, Mencken T *et al.* (2012) Discovery of 7-methyl-5-(1-{{3-(trifluoromethyl)phenyl}acetyl}-2,3-dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). *J Med Chem* **55**, 7193–7207.

- 316 Moreno JA, Halliday M, Molloy C, Radford H, Verity N, Axten JM, Ortori CA, Willis AE, Fischer PM, Barrett DA *et al.* (2013) Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Sci Transl Med* **138**, 206ra138.
- 317 Harding HP, Zeng H, Zhang Y, Jungries R, Chung P, Plesken H, Sabatini DD & Ron D (2001) Diabetes mellitus and exocrine pancreatic dysfunction in *perk*<sup>-/-</sup> mice reveals a role for translational control in secretory cell survival. *Mol Cell* **7**, 1153–1163.
- 318 Rojas-Rivera D, Delvaeye T, Roelandt R, Nerinckx W, Augustyns K, Vandenaebelle P & Bertrand MJ (2017) When PERK inhibitors turn out to be new potent RIPK1 inhibitors: critical issues on the specificity and use of GSK2606414 and GSK2656157. *Cell Death Differ* **24**, 1100–1110.
- 319 Sidrauski C, Tsai JC, Kampmann M, Hearn BR, Vedantham P, Jaishankar P, Sokabe M, Mendez AS, Newton BW, Tang EL *et al.* (2015) Pharmacological dimerization and activation of the exchange factor eIF2B antagonizes the integrated stress response. *Elife* **2015**, e07314.
- 320 Sekine Y, Zyryanova A, Crespillo-Casado A, Fischer PM, Harding HP & Ron D (2015) Stress responses. Mutations in a translation initiation factor identify the target of a memory-enhancing compound. *Science* **348**, 1027–1030.
- 321 Halliday M, Radford H, Sekine Y, Moreno J, Verity N, Le Quesne J, Ortori CA, Barrett DA, Fromont C, Fischer PM *et al.* (2015) Partial restoration of protein synthesis rates by the small molecule ISRIB prevents neurodegeneration without pancreatic toxicity. *Cell Death Dis* **6**, e1672.
- 322 Sidrauski C, McGeachy AM, Ingolia NT & Walter P (2015) The small molecule ISRIB reverses the effects of eIF2 $\alpha$  phosphorylation on translation and stress granule assembly. *Elife* **2015**, 1–16.
- 323 Gallagher CM, Garri C, Cain EL, Ang KKH, Wilson CG, Chen S, Hearn BR, Jaishankar P, Aranda-Diaz A, Arkin MR *et al.* (2016) Ceapins are a new class of unfolded protein response inhibitors, selectively targeting the ATF6 $\alpha$  branch. *Elife* **5**, 1–33.
- 324 Gallagher CM & Walter P (2016) Ceapins inhibit ATF6 $\alpha$  signaling by selectively preventing transport of ATF6 $\alpha$  to the Golgi apparatus during ER stress. *Elife* **5**, e11880.
- 325 Bu L, Yu H, Fan L, Li X, Wang F, Liu J, Zhong F, Zhang C, Wei W, Wang H *et al.* (2017) Melatonin, a novel selective ATF-6 inhibitor, induces human hepatoma cell apoptosis through COX-2 downregulation. *World J Gastroenterol* **23**, 986–998.
- 326 Xu S, Butkevich AN, Yamada R, Zhou Y, Debnath B, Duncan R, Zandi E, Petasis NA & Neamati N (2012) Discovery of an orally active small-molecule irreversible inhibitor of protein disulfide isomerase for ovarian cancer treatment. *Proc Natl Acad Sci* **109**, 16348–16353.
- 327 Banerjee R, Pace NJ, Brown DR & Weerapana E (2013) 1,3,5-Triazine as a modular scaffold for covalent inhibitors with streamlined target identification. *J Am Chem Soc* **135**, 2497–2500.
- 328 Chevet E, Hetz C & Samali A (2015) Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. *Cancer Discov* **5**, 586–597.
- 329 Okada T, Haze K, Nadanaka S, Yoshida H, Seidah NG, Hirano Y, Sato R, Negishi M & Mori K (2003) A serine protease inhibitor prevents endoplasmic reticulum stress-induced cleavage but not transport of the membrane-bound transcription factor ATF6. *J Biol Chem* **278**, 31024–31032.
- 330 Doultzinos D, Avril T, Lhomond S, Dejeans N, Guédat P & Chevet E (2017) Control of the unfolded protein response in health and disease. *SLAS Discov* **22**, 787–800.
- 331 Delpino A & Castelli M (2002) The 78 kDa glucose-regulated protein (GRP78/BIP) is expressed on the cell membrane, is released into cell culture medium and is also present in human peripheral circulation. *Biosci Rep* **22**, 407–420.
- 332 Chessler SD & Byers PH (1993) BiP binds type I procollagen pro alpha chains with mutations in the carboxyl-terminal propeptide synthesized by cells from patients with osteogenesis imperfecta. *J Biol Chem* **268**, 18226–18233.
- 333 Booth L, Roberts JL, Cash DR, Tavallai S, Jean S, Fidanza A, Cruz-Luna T, Siembiba P, Cycon KA, Cornelissen CN *et al.* (2015) GRP78/BiP/HSPA5/DNA K is a universal therapeutic target for human disease. *J Cell Physiol* **230**, 1661–1676.
- 334 Zhu H, Chen X, Chen B, Chen B, Song W, Sun D & Zhao Y (2014) Activating transcription factor 4 promotes esophageal squamous cell carcinoma invasion and metastasis in mice and is associated with poor prognosis in human patients. *PLoS ONE* **9**, 1–11.
- 335 Yang J, Cheng D, Zhou S, Zhu B, Hu T & Yang Q (2015) Overexpression of X-Box binding protein 1 (XBP1) correlates to poor prognosis and up-regulation of PI3K/mTOR in human osteosarcoma. *Int J Mol Sci* **16**, 28635–28646.
- 336 Bujisic B, De Gassart A, Tallant R, Demaria O, Zaffalon L, Chelbi S, Gilliet M, Bertoni F & Martinon F (2017) Impairment of both IRE1 expression and XBP1 activation is a hallmark of GCB DLBCL and contributes to tumor growth. *Blood* **129**, 2420–2428.
- 337 Kaser A, Lee A-H, Franke A, Glickman JN, Zeissig S, Tilg H, Nieuwenhuis EES, Higgins DE, Schreiber S,

- Glimcher LH *et al.* (2008) XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* **134**, 743–756.
- 338 Atkin JD, Farg MA, Walker AK, McLean C, Tomas D & Horne MK (2008) Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. *Neurobiol Dis* **30**, 400–407.
- 339 Nardo G, Pozzi S, Pignataro M, Lauranzano E, Spano G, Garbelli S, Mantovani S, Marinou K, Papetti L, Monteforte M *et al.* (2011) Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. *PLoS ONE* **6**, e25545.
- 340 Kim Y, Lee H, Manson SR, Lindahl M, Evans B, Miner JH, Urano F & Chen YM (2016) Mesencephalic astrocyte-derived neurotrophic factor as a urine biomarker for endoplasmic reticulum stress-related kidney diseases. *J Am Soc Nephrol* **27**, 2974–2982.
- 341 Mami I, Bouvier N, El Karoui K, Gallazzini M, Rabant M, Laurent-Puig P, Li S, Tharaux PL, Beaune P, Thervet E *et al.* (2016) Angiogenin mediates cell-autonomous translational control under endoplasmic reticulum stress and attenuates kidney injury. *J Am Soc Nephrol* **27**, 863–876.
- 342 Dadey DYA, Kapoor V, Hoyer K, Khudanyan A, Collins A, Thotala D & Hallahan DE (2016) Antibody targeting GRP78 enhances the efficacy of radiation therapy in human glioblastoma and non-small-cell lung cancer cell lines and tumor models. *Clin Cancer Res* **23**, 2556–2564.
- 343 Lin YG, Shen J, Yoo E, Liu R, Yen HY, Mehta A, Rajaei A, Yang W, Mhawech-Fauceglia P, DeMayo FJ *et al.* (2015) Targeting the glucose-regulated protein-78 abrogates Pten-null driven AKT activation and endometrioid tumorigenesis. *Oncogene* **34**, 5418–5426.
- 344 Chen YG, Ashok BT, Liu X, Garikapaty VPS, Mittelman A & Tiwari RK (2003) Induction of heat shock protein gp96 by immune cytokines. *Cell Stress Chaperones* **8**, 242–248.
- 345 Zhang K, Peng Z, Huang X, Qiao Z, Wang X, Wang N, Xi H, Cui J, Gao Y, Huang X *et al.* (2017) Phase II trial of adjuvant immunotherapy with autologous tumor-derived Gp96 vaccination in patients with gastric cancer. *J Cancer* **8**, 1826–1832.
- 346 Tian S, Liu Z, Donahue C, Falo LD Jr & You Z (2012) Genetic targeting of the active transcription factor XBPs to dendritic cells potentiates vaccine-induced prophylactic and therapeutic antitumor immunity. *Mol Ther* **20**, 432–442.
- 347 Miller M, Rosenthal P, Beppu A, James L, Hoffman HM, Tam AB, Taylor A, McGeough MD, Pena CA, Niwa M *et al.* (2014) ORMDL3 transgenic mice have increased airway remodeling and airway responsiveness characteristic of asthma. *J Immunol* **192**, 3475–3487.
- 348 Graner MW, Lillehei KO & Katsanis E (2015) Endoplasmic reticulum chaperones and their roles in the immunogenicity of cancer vaccines. *Front Oncol* **4**, 1–12.
- 349 Graner MW, Zeng Y, Feng H & Katsanis E (2003) Tumor-derived chaperone-rich cell lysates are effective therapeutic vaccines against a variety of cancers. *Cancer Immunol Immunother* **52**, 226–234.
- 350 Rapp UK & Kaufmann SH (2004) DNA vaccination with gp96-peptide fusion proteins induces protection against an intracellular bacterial pathogen. *Int Immunol* **16**, 597–605.
- 351 Qian J, Hong S, Wang S, Zhang L, Sun L, Wang M, Yang J & Kwak LW (2009) Myeloma cell line – derived, pooled heat shock proteins as a universal vaccine for immunotherapy of multiple myeloma. *Blood* **114**, 3880–3890.
- 352 Argon Y & Simen BB (1999) GRP94, an ER chaperone with protein and peptide binding properties. *Semin Cell Dev Biol* **10**, 495–505.
- 353 Liu D, Liu X, Zhou T, Yao W, Zhao J, Zheng Z, Jiang W, Wang F, Aikhionbare FO, Hill DL *et al.* (2015) IRE1-RACK1 axis orchestrates ER stress preconditioning-elicited cytoprotection from ischemia/reperfusion injury in liver. *J Mol Cell Biol* **8**, 144–156.
- 354 Bailly-Maitre B, Fondevila C, Kaldas F, Droin N, Luciano F, Ricci J-E, Croxton R, Krajewska M, Zapata JM, Kupiec-Weglinski JW *et al.* (2006) Cytoprotective gene bi-1 is required for intrinsic protection from endoplasmic reticulum stress and ischemia-reperfusion injury. *Proc Natl Acad Sci USA* **103**, 2809–2814.
- 355 Bi X, Zhang G, Wang X, Nguyen C, May HI, Li X, Al-Hashimi AA, Austin RC, Gillette TG, Fu G *et al.* (2018) Endoplasmic reticulum chaperone GRP78 protects heart from ischemia/reperfusion injury through Akt activation. *Circ Res* **122**, 1545–1554.
- 356 Martindale JJ, Fernandez R, Thuerlauf D, Whittaker R, Gude N, Sussman MA & Glembotski CC (2006) Endoplasmic reticulum stress gene induction and protection from ischemia/reperfusion injury in the hearts of transgenic mice with a tamoxifen-regulated form of ATF6. *Circ Res* **98**, 1186–1193.
- 357 Peralta C & Brenner C (2011) Endoplasmic reticulum stress inhibition enhances liver tolerance to ischemia/reperfusion. *Curr Med Chem* **18**, 2016–2024.
- 358 Folch-Puy E, Panisello A, Oliva J, Lopez A, Benítez CC, Adam R & Roselló-Catafau J (2016) Relevance of endoplasmic reticulum stress cell signaling in liver cold ischemia reperfusion injury. *Int J Mol Sci* **17**, 1–12.
- 359 Kwon SK, Ahn M, Song HJ, Kang SK, Jung SB, Harsha N, Jee S, Moon JY, Suh KS, Do LS *et al.* (2015) Nafamostat mesilate attenuates transient focal

- ischemia/reperfusion-induced brain injury via the inhibition of endoplasmic reticulum stress. *Brain Res* **1627**, 12–20.
- 360 Zhang H, Yue Y, Sun T, Wu X & Xiong S (2017) Transmissible endoplasmic reticulum stress from myocardiocytes to macrophages is pivotal for the pathogenesis of CVB3-induced viral myocarditis. *Sci Rep* **7**, 42162.
- 361 Rodvold JJ, Chiu KT, Hiramatsu N, Nussbacher JK, Galimberti V, Mahadevan NR, Willert K, Lin JH & Zanetti M (2017) Intercellular transmission of the unfolded protein response promotes survival and drug resistance in cancer cells. *Sci Signal* **10**, eaah7177. <https://doi.org/10.1126/scisignal.aah7177>
- 362 Brownlie RJ, Myers LK, Wooley PH, Corrigan VM, Bodman-Smith MD, Panayi GS & Thompson SJ (2006) Treatment of murine collagen-induced arthritis by the stress protein BiP Via interleukin-4 – producing regulatory t cells a novel function for an ancient protein. *Arthritis Rheumatol* **54**, 854–863.
- 363 Kirkham B, Chaabo K, Hall C, Garrood T, Mant T, Allen E, Vincent A, Vasconcelos JC, Prevost AT, Panayi GS *et al.* (2016) Safety and patient response as indicated by biomarker changes to binding immunoglobulin protein in the phase I/IIA RAGULA clinical trial in rheumatoid arthritis. *Rheumatology (United Kingdom)* **55**, 1993–2000.
- 364 Axten JM, Romeril SP, Shu A, Ralph J, Medina JR, Feng Y, Li WHH, Grant SW, Heering DA, Minthorn E *et al.* (2013) Discovery of GSK2656157: an optimized perk inhibitor selected for preclinical development. *ACS Med Chem Lett* **4**, 964–968.
- 365 Boyce M, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, Kaufman RJ, Ma D, Coen DM, Ron D *et al.* (2005) A selective inhibitor of eIF2 $\alpha$  dephosphorylation protects cells from ER stress. *Science* **307**, 935–939.
- 366 Saxena S, Cabuy E & Caroni P (2009) A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. *Nat Neurosci* **12**, 627–636.
- 367 Colla E, Coune P, Liu Y, Pletnikova O, Troncoso JC, Iwatsubo T, Schneider BL & Lee MK (2012) Endoplasmic reticulum stress is important for the manifestations of  $\alpha$ -synucleinopathy *in vivo*. *J Neurosci* **32**, 3306–3320.
- 368 Wang L, Popko B, Tixier E & Roos RP (2014) Guanabenz, which enhances the unfolded protein response, ameliorates mutant SOD1-induced amyotrophic lateral sclerosis. *Neurobiol Dis* **71**, 317–324.
- 369 Das I, Krzyzosiak A, Schneider K, Wrabetz L, D'Antonio M, Barry N & Sigurdardottir ABA (2015) Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science* **348**, 239–242.
- 370 Lee A-H, Iwakoshi NN & Glimcher LH (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* **23**, 7448–7459.
- 371 Ghosh R, Wang L, Wang ES, Perera BGK, Igarria A, Morita S, Prado K, Thamsen M, Caswell D, Macias H *et al.* (2014) Allosteric inhibition of the IRE1 $\alpha$  RNase preserves cell viability and function during endoplasmic reticulum stress. *Cell* **158**, 534–548.
- 372 Kawamura T, Tashiro E, Shindo K & Imoto M (2008) SAR study of a novel triene-ansamycin group compound, quinotrierixin, and related compounds, as inhibitors of ER stress-induced XBP1 activation II. Structure elucidation. *J Antibiot (Tokyo)* **61**, 312–317.
- 373 Chen D, Landis-Piowar KR, Chen MS & Dou QP (2007) Inhibition of proteasome activity by the dietary flavonoid apigenin is associated with growth inhibition in cultured breast cancer cells and xenografts. *Breast Cancer Res* **9**, R80.
- 374 Zhu M, Rajamani S, Kaylor J, Han S, Zhou F & Fink AL (2004) The flavonoid baicalein inhibits fibrillation of alpha-synuclein and disaggregates existing fibrils. *J Biol Chem* **279**, 26846–26857.
- 375 Kim D-S, Ha K-C, Kwon D-Y, Kim M-S, Kim H-R, Chae S-W & Chae H-J (2008) Kaempferol protects ischemia/reperfusion-induced cardiac damage through the regulation of endoplasmic reticulum stress. *Immunopharmacol Immunotoxicol* **30**, 257–270.
- 376 Plate L, Cooley CB, Chen JJ, Paxman RJ, Gallagher CM, Madoux F, Genereux JC, Dobbs W, Garza D, Spicer TP *et al.* (2016) Small molecule proteostasis regulators that reprogram the ER to reduce extracellular protein aggregation. *Elife* **5**, e15550.
- 377 Higa A, Taouji S, Lhomond S, Jensen D, Fernandez-Zapico ME, Simpson JC, Pasquet JM, Schekman R & Chevet E (2014) Endoplasmic reticulum stress-activated transcription factor atf6 $\alpha$  requires the disulfide isomerase PDIA5 to modulate chemoresistance. *Mol Cell Biol* **34**, 1839–1849.