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Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications

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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...
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 Ca²⁺ 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 Ca²⁺ 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
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 isomerase (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).

Ca²⁺ homeostasis

Ca²⁺ 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 Ca²⁺ storage, plays a pivotal role in the regulation of Ca²⁺ levels and reciprocally many ER functions are controlled in a Ca²⁺-dependent way, thereby regulating the calcium homeostasis of the whole cell [34]. Consequently, both ER and cytosolic Ca²⁺ concentrations need to be highly spatiotemporally regulated in order for the ER to maintain a much increased physiological intraluminal Ca²⁺ concentration and oxidizing redox potential than the cytoplasm. To modulate these levels, the ER employs a number of mechanisms that control Ca²⁺ concentration on both sides of the membrane: (a) ER membrane ATP-dependent Ca²⁺ pumps for cytosol-to-lumen transport; (b) ER luminal Ca²⁺-binding chaperones for sequestering free Ca²⁺; and (c) ER membrane channels for the regulated release of Ca²⁺ 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 the Philadelphia chromosome, BCR-ABL1, is a constitutively active oncoprotein that enhances cell proliferation and interferes with Ca\(^{2+}\)-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 β 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\(^{2+}\)-ATPase (SERCA) with compounds, such as thapsigargin and cyclopiazonic acid [54,55], induces ER stress by reducing ER Ca\(^{2+}\) 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, Ca²⁺, ER oxidooreductin 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 (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 IRE1α [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 (IRE1α and β), 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]. IRE1α (referred to IRE1 hereafter) is ubiquitously expressed; however, inositol-requiring enzyme 1 β (IRE1β) 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 Ern2WT 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.
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

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.
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 (ERα) [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) versus 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 exonucleases [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, IRE1β was found to selectively induce translational repression through the 28S ribosomal RNA cleavage [81] demonstrating that IRE1α and IRE1β 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 nondepressible 2, can phosphorylate eIF2α [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 eIF2α on serine 51 [150]. eIF2α 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, eIF2α 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]. eIF2α
phosphorylation (p-eIF2α) 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 eIF2α 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 eIF2α thereby creating a negative feedback loop that antagonizes p-eIF2α-dependent translation inhibition and restores protein synthesis [169,170,172]. The translational arrest induced by p-eIF2α 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 eIF2α 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 ATF6α [181] and ATF6B for ATF6β [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], ATF6β 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 ATF6α [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 organisal 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 activate genes involved in protein folding, processing, and degradation [185]. ATF4, activated downstream of PERK and p-eIF2α, 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 (eIF2α) 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 described 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 Ca2+ 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 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 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 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-α, resulting in ER Ca$^{2+}$ release and apoptosis in macrophages [204]. Direct interactions of PDIs with ER stress sensors, protein S-nitrosylation and ER 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–eIF2α pathway has been reported to regulate lipogenesis and hepatic steatosis. Compromising eIF2α phosphorylation in mice by overexpression of GADD34 results in reduced hepatosteatosis upon high-fat diet [212]. ATF4 the downstream effector of PERK–eIF2α 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–eIF2α 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 β-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 β-cell-specific XBP1 mutant mice show hyperglycaemia and glucose intolerance due to decreased insulin release of β-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, PPARα, 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 prosurvival 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 prosurvival to prodeath. 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-eIF2α 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 prosurvival, 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 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]. IRE1α 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 Ca2+ 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 eIF2α 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 ATF6α 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 oyster ethanol 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/EBPα, transcriptionally downregulating 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 XBP1 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 knockdown 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 trophectoderm 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 β cell architecture but not in cell death, and was accompanied by an increase in β 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 1 collagen is an OASIS target [285]. Moreover, osteoblast OASIS expression is controlled by factors essential to osteogenesis (BMP2), pointing to a PERK-eIF2α-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–eIF2α 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 UPRE-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
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 5HGI), as a back-to-back dimer. Notably, the DFG motif (Asp711-Phe712-Gly713) faces into the active site (DFG-in), with helix-aC-in conformation. In contrast, human IRE1 bound to KIRA compound 33 (PDB: 4U6R) shows an inactive kinase conformation, with DFG-in and helix-aC-out conformation. The inactive conformation is incompatible with back-to-back dimer formation due to the displaced helix-aC [301]. Imidazopyrazine-based inhibitors and other KIRAs

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).

<table>
<thead>
<tr>
<th>UPR Arm</th>
<th>Name</th>
<th>Target</th>
<th>Brief description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>PERK</td>
<td>GSK2656157</td>
<td>PERK Kinase</td>
<td>In preclinical stage for multiple myeloma and pancreatic cancer</td>
<td>[314,364]</td>
</tr>
<tr>
<td>Salubrinal</td>
<td>GADD34/PP1c</td>
<td>Inhibition of eIF2a dephosphorylation</td>
<td>In ALS, it increases lifespan of mutant superoxide dismutase 1 transgenic mice</td>
<td>[365–367]</td>
</tr>
<tr>
<td></td>
<td>ISRIB</td>
<td>eIF2β</td>
<td>Decreased ATF4 expression</td>
<td>[322]</td>
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<tr>
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<td>Guanabenz</td>
<td>GADD34/PP1c</td>
<td>Inhibitor of eIF2α phosphatase</td>
<td>[368]</td>
</tr>
<tr>
<td></td>
<td>Sephin1</td>
<td>GADD34 (FP1c)</td>
<td>Inhibitor of eIF2α phosphatase</td>
<td>[369]</td>
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<tr>
<td>IRE1</td>
<td>Salicyldimines</td>
<td>IRE1 RNase</td>
<td>IRE1α RNAse active-site inhibitor</td>
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<tr>
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<td>STF-083010</td>
<td>IRE1 RNase</td>
<td>In preclinical stage for multiple myeloma treatment</td>
<td>[308]</td>
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<tr>
<td></td>
<td>KIRA</td>
<td>IRE1 Kinase</td>
<td>IRE1α kinase active-site inhibitor</td>
<td>[371]</td>
</tr>
<tr>
<td></td>
<td>Toyocamycin</td>
<td>IRE1 RNase</td>
<td>IRE1α RNAse active-site inhibitor</td>
<td>[309,372]</td>
</tr>
<tr>
<td></td>
<td>3-ethoxy-5,6-</td>
<td>IRE1 RNase</td>
<td>IRE1α RNAse active-site inhibitor</td>
<td>[305]</td>
</tr>
<tr>
<td></td>
<td>dibromosalicylaldehyde</td>
<td></td>
<td>In preclinical stage for various cancers treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apigenin</td>
<td>Proteasome</td>
<td>Increase of IRE1α nuclease activity in model</td>
<td>[373]</td>
</tr>
<tr>
<td></td>
<td>FIRE peptide</td>
<td>IRE1 Kinase</td>
<td>Modulation IRE1 oligomerization in vitro, Xbp1 mRNA cleavage in vitro, in cell culture and in vivo (Caenorhabditis elegans)</td>
<td>[85]</td>
</tr>
<tr>
<td>ATF6</td>
<td>Apigenin</td>
<td>ATF6</td>
<td>Upregulation of ATF6 expression</td>
<td>[373]</td>
</tr>
<tr>
<td></td>
<td>Baicalein</td>
<td>ATF6</td>
<td>Upregulation of ATF6 expression</td>
<td>[374]</td>
</tr>
<tr>
<td></td>
<td>Ceapin</td>
<td>ND</td>
<td>Inhibitor of ATF6</td>
<td>[323]</td>
</tr>
<tr>
<td></td>
<td>Kaempferol</td>
<td>ATF6</td>
<td>Downregulation of ATF6 expression</td>
<td>[375]</td>
</tr>
<tr>
<td></td>
<td>Melatonin</td>
<td>ATF6</td>
<td>Inhibitor of ATF6</td>
<td>[325]</td>
</tr>
<tr>
<td></td>
<td>Compound 147</td>
<td>ATF6</td>
<td>Activator of ATF6</td>
<td>[376]</td>
</tr>
<tr>
<td></td>
<td>Compound 263</td>
<td>ATF6</td>
<td>Activator of ATF6</td>
<td>[376]</td>
</tr>
<tr>
<td></td>
<td>16F16</td>
<td>PDI</td>
<td>Inhibitor of PDI</td>
<td>[377]</td>
</tr>
</tbody>
</table>

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

IRE1 RNase-binding site
IRE1 RNase inhibitors include salicylaldehydes [305] 4m8C [306], MKC-946 [307], STF-83010 [308], toyocamycin [309] and hydroxyl-aryl-aldehydes [86]. The reported co-crystal structures of murine IRE1α 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 IRE1α-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 XBPI 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-eIF2α 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−/− mice [317]. Furthermore, GSK2606414 and GSK2656157 were found recently to inhibit RIPK1 at nanomolar concentrations [318]. To overcome the β-cell toxicity, small molecules modulating the eIF2α 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
challenges. 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 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 prodrug 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 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 chaperons 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, toyoacymycin 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
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>
<thead>
<tr>
<th>Trial</th>
<th>Disease</th>
<th>Intervention</th>
<th>Country</th>
</tr>
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<tbody>
<tr>
<td>Role of ER stress in the pathophysiology of type 2 diabetes</td>
<td>Diabetes mellitus, type 2</td>
<td>No intervention</td>
<td>France</td>
</tr>
<tr>
<td>ER stress and resistance to treatments in Ph-negative myeloproliferative neoplasms</td>
<td>Polycythemia vera</td>
<td>Biological: RNA sample of total leucocytes before start of treatment</td>
<td>France</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Drug: TUDCA</td>
<td>United States</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>Other: placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>Drug: sodium phenylbutyrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect of ER stress on metabolic function</td>
<td>Chronic airway disorders</td>
<td>Observational</td>
<td>South Korea</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Drug: TUDCA</td>
<td></td>
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</tr>
<tr>
<td>TUDCA for protease-inhibitor associated insulin resistance</td>
<td>HIV-related insulin resistance</td>
<td>Drug: TUDCA</td>
<td>United States</td>
</tr>
<tr>
<td>Protease inhibitor-related Insulin resistance</td>
<td>Other: placebo tablet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER stress in NAFLD</td>
<td>Obesity</td>
<td>Drug: methyl-D9-choline</td>
<td>United States</td>
</tr>
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<td>NAFLD</td>
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<tr>
<td>TUDCA in new-onset type 1 diabetes</td>
<td>Type 1 diabetes</td>
<td>Drug: TUDCA</td>
<td>United States</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>Drug: Sugar Pill (placebo)</td>
<td></td>
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<tr>
<td>Effects of Liraglutide on ER stress in obese patients with type 2 diabetes</td>
<td>Wolfram syndrome</td>
<td>Drug: dantrolene sodium</td>
<td>United States</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Drug: liraglutide</td>
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<tr>
<td>optic nerve atrophy</td>
<td>Drug: dantrolene sodium</td>
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<tr>
<td>Ataxia</td>
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</table>

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.
Box 1. First-in-human trial
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-/- mice were reported to have more prominent increase in NF-kB activation and further upregulation of proinflammatory genes (CXCL-1, MIP-2, IL-6) [342]. Interestingly, inhibition of IRE1-NF-kB 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 exerts in interorgan communication affecting disease. In particular, it appears an indispensable tool 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].

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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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