Proteostasis control by the unfolded protein response

Claudio Hetz, Eric Chevet and Scott A. Oakes

Stress induced by accumulation of misfolded proteins in the endoplasmic reticulum is observed in many physiological and pathological conditions. To cope with endoplasmic reticulum stress, cells activate the unfolded protein response, a dynamic signalling network that orchestrates the recovery of homeostasis or triggers apoptosis, depending on the level of damage. Here we provide an overview of recent insights into the mechanisms that cells employ to maintain proteostasis and how the unfolded protein response determines cell fate under endoplasmic reticulum stress.

The endoplasmic reticulum (ER) orchestrates the synthesis, folding and structural maturation of at least a third of all proteins in the cell. Most proteins that are ultimately secreted or reside in the ER, Golgi apparatus, lysosomes and plasma membrane are translated on ER membrane-associated ribosomes and transported into the ER lumen. As such, the ER is a key contributor to proteostasis — a network of interconnected quality-control processes in the cell that maintains the functional proteome[1]. Chaperones, oxidoreductases and glycosylating enzymes ensure that secretory proteins are properly folded, modified and assembled into multi-protein complexes in the ER before they transit farther downstream in the secretory pathway. Despite the existence of these protein-folding machines, there is evidence to suggest that at least a third of all polypeptides translocated into the ER fail to satisfy the quality-control mechanisms that ensure proper folding, and for some proteins the success rate is much lower[2]. Given that client proteins of the secretory pathway often carry out crucial signalling functions (for example, polypeptide hormones and cell surface receptors), improperly folded forms are recognized and disposed of by stringent quality-control systems such as ER-associated degradation (ERAD), which removes unfolded proteins to the cytosol for subsequent ubiquitylation and degradation by the 26S proteasome. Moreover, inherited mutations in an individual protein can compromise its folding efficiency and lead to disease from its resulting deficiency. For example, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), an ion channel transporting chloride across epithelial tissues, disrupt its folding within the ER, leading to cystic fibrosis.

The protein-folding capacity within the ER differs widely among cell types. Specialized secretory cells contain a large, well-developed ER. For example, each β-cell of the endocrine pancreas is capable of synthesizing and secreting up to one million molecules of insulin per minute; in insulin-resistant states, this enormous protein synthetic load becomes even greater[3]. Regardless of the size of their ER, cells seem to perform near the functional limits of their secretory pathway capacity and often face conditions during which the load imposed on the ER protein-folding machinery overwhelms capability, a condition referred to as ER stress. A wide range of cellular perturbations can induce ER stress, including hypoxia, nutrient deprivation, point mutations in secreted proteins that promote misfolding, redox changes and loss of calcium homeostasis with harmful effects to ER-resident calcium-dependent chaperones. Therefore, cells have evolved a robust surveillance system to respond to fluctuations of ER homeostasis before they become a threat to survival.

ER stress engages an adaptive signal transduction pathway called the unfolded protein response (UPR). However, irremediable ER stress turns signalling toward a 'terminal UPR' that drives cells into apoptosis. Cell injury due to chronic ER stress is increasingly being recognized as a common contributor to a wide range of prevalent human diseases, including neurodegeneration, diabetes, cancer, stroke, pulmonary fibrosis, viral infections, inflammation , metabolic disorders, and heart disease[4]. Shared among these seemingly dissimilar diseases is the presence of intracellular and/or extracellular conditions that compromise protein folding and cause ER stress. Here we discuss recent advances in our understanding of how the UPR network maintains proteostasis in healthy cells, but actively promotes cell loss when ER homeostasis cannot be restored.

Key players in the UPR

The discovery of an ER stress-induced adaptive response in the yeast *Saccharomyces cerevisiae* in the late 1980s[5–7], led to much speculation about a potential role for a UPR pathway in mammals. The field

Claudio Hetz is at the Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Santiago, Chile; the Institute of Biomedical Sciences (ICBM), Program of Cellular and Molecular Biology, Center for Molecular Studies of the Cell, University of Chile, Santiago, Chile; and in the Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts 02115, USA. Eric Chevet is at ER440, Oncogenesis, stress and signaling, University of Rennes 1, 35000 Rennes, France; and in the Centre Régional de Lutte Contre le Cancer Eugène Marquis, 35000 Rennes, France. Scott A. Oakes is in the Department of Pathology, Diabetes Center, and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, California 94143, USA.
e-mail: chetz@med.uchile.cl; eric.chevet@inserm.fr; scott.oakes@ucsf.edu
Published online 30 June 2015; corrected after print 13 July 2015; DOI: 10.1038/ncb3184

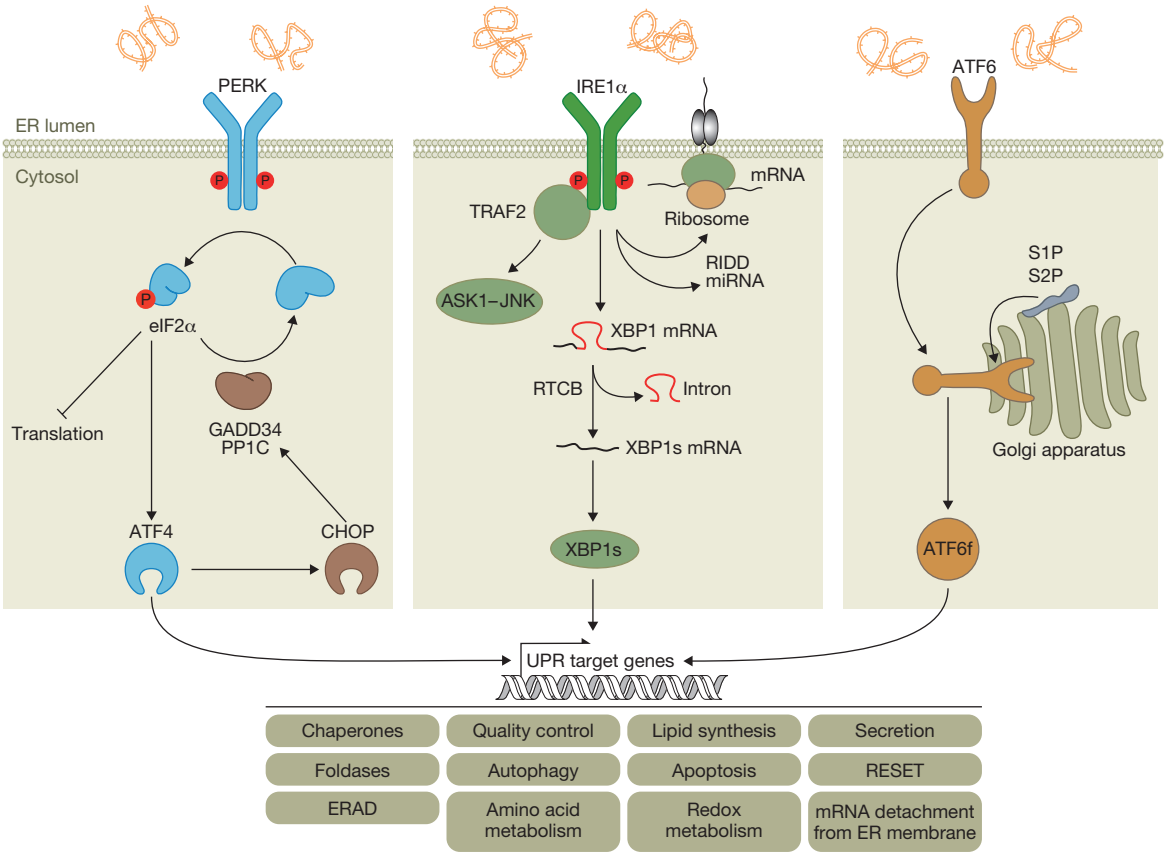

Figure 1 The three arms of the UPR. All three ER stress sensors (PERK, IRE1α, ATF6) are present in the ER membrane and initially activate signalling events that increase protein-folding capacity and reduce protein load on the ER. These transcriptional and translational outputs tend to re-establish protein-folding homeostasis in the ER and promote cell survival. PERK phosphorylates eIF2α, which in turn shuts down global translation and concomitantly increases the expression of the transcription factor ATF4. The latter induces the transcription of select genes whose functions are to restore proteostasis and of CHOP, itself inducing the transcription of GADD34, a regulatory subunit of PP1C. This creates a feedback mechanism leading to the dephosphorylation of eIF2α and translation is reinitiated. IRE1α signals through (i) the recruitment of TRAF2 leading the activation of the ASK1–JNK cascade and (ii) through its RNase via the splicing of XBP1 mRNA or the degradation of RNAs (RIDD activity), thereby regulating gene expression at transcriptional and post-transcriptional levels. Finally, following ER stress, ATF6 is exported from the ER to the Golgi complex where it is cleaved by the proteases S1P and S2P, releasing its cytosolic domain which is a potent transcription factor. Together, UPR transcription factors determine cell fate by the regulation of distinct subsets of target genes toward recovery of ER homeostasis or the induction of apoptosis. The grey boxes illustrate the different functions of genes induced by the ER stress response. RESET, rapid ER stress induced export.

exploded with the discovery of the three main mammalian UPR sensors IRE1α (endoribonuclease inositol-requiring enzyme 1-alpha), PERK (protein kinase RNA-like endoplasmic reticulum kinase) and ATF6α (activating transcription factor 6) in 1993[8], 1998[9] and 1999[10], respectively. The signalling pathways activated downstream of these sensors constitute an adaptive response to allow cells to cope with protein misfolding by temporarily reducing *de novo* protein synthesis, and improving the folding and clearance capacity of the ER[11]. However, if these adaptive measures are inadequate to resolve ER stress, the mammalian UPR shifts signals toward the engagement of apoptosis[12]. In this section we discuss the main signalling mechanisms involved in the UPR and the downstream consequences in terms of the cellular processes affected.

Detection of misfolded protein species by the three UPR sensors is partly dependent on BiP (also known as GRP78), a key ER chaperone. Under basal conditions, BiP constitutively binds to the luminal domains of the three sensors thus preventing their activation. However, when misfolded proteins accumulate in the ER, BiP strongly binds to their exposed hydrophobic domains, and dissociates from the UPR sensors, thereby priming IRE1α and PERK for oligomerization and auto-transphosphorylation[13], and revealing an ER export motif in ATF6α (ref. 14). BiP binding to UPR sensors was recently shown to be independent of its chaperone activity, suggesting an allosteric regulation[15]. The analysis of the ER luminal structure of IRE1p in *S. cerevisiae* revealed an MHC-like structure[16] that appears to serve as a direct sensing motif that binds to hydrophobic domains of unfolded proteins[17]. A similar model has been proposed[18] for IRE1β, but may not operate for IRE1α based on structural and *in vitro* analyses[15,19–21].

Stress signalling leads to selective activation of downstream cascades (Fig. 1), in which active PERK phosphorylates the translation initiation factor eIF2α leading to attenuation of global protein synthesis[22,23]. Inhibition of eIF2α allows the selective expression of ATF4, a transcription factor controlling genes involved in protein folding, antioxidant responses, autophagy, amino acid metabolism and apoptosis[24,25]. Moreover, active PERK phosphorylates and activates NRF2, a transcription factor involved in the control of the antioxidant pathway[26–28]. Finally, ATF6α belongs to a family of transmembrane transcription

factors of about 10 members that function in different ways as stress response elements[29]. Following ER stress, ATF6α is exported from the ER (ref. 10) and activated in the Golgi complex through cleavage by the proteases S1P and S2P (ref. 11). The ATF6α cytosolic domain (ATF6f) then translocates to the nucleus where it activates specific transcriptional programs that promote adaptation, including upregulation of ERAD components[30].

Striking advances in understanding IRE1α signalling have been made recently. IRE1α is an ER transmembrane protein with kinase and endoribonuclease (RNase) activities associated with its cytoplasmic tail. In response to luminal activation, IRE1α dimerizes and transautophosphorylates, inducing a conformational change that activates the RNase domain. IRE1α's RNase catalyses the excision of a 26-nucleotide intron within the XBP1 mRNA, shifting the reading frame to translate a stable and active transcription factor known as XBP1s (refs 31–34). XBP1s controls genes involved in protein folding, secretion, ERAD and lipid synthesis[35–37]. In addition, XBP1s may heterodimerize with ATF6f to control distinct gene expression patterns[38]. The unspliced XBP1u protein may regulate the efficient delivery of its mRNA to the ER for processing[39,40], in addition to controlling the degradation of XBP1s[41,42]. IRE1α is involved in the degradation of RNAs (known as regulated IRE1-dependent decay or RIDD)[43], including ER-localized mRNAs[44–46], ribosomal RNA[47] and microRNAs[48,49]. New insights into the mechanisms regulating IRE1α's switch from XBP1 mRNA splicing to RIDD have been recently reported. IRE1α's RNase substrate specificity can be controlled by its oligomeric state[50]. When IRE1α exists in lower order oligomers (for example, dimers/tetramers), its RNase activity is largely restricted to XBP1 splicing. However, under high or chronic ER stress, IRE1α surpasses an oligomerization threshold that expands its RNase substrate repertoire to many ER-localized mRNAs, leading to RIDD. In contrast to this view, a recent study suggested that XBP1 mRNA splicing requires obligate IRE1α oligomers, but that IRE1α dimers suffice for RIDD (ref. 51). This is consistent with the idea that RIDD activity can be observed at basal levels in certain systems[43]. Of note, this later report was largely restricted to *in vitro* analyses of IRE1α activity under saturating protein concentrations, which do not mimic the continuum of IRE1α oligomerization states that likely exist *in vivo*. However, this model is also supported by cell-based studies in which peptide-induced IRE1 high order oligomers correlated with enhanced IRE1-mediated XBP1 mRNA splicing and reduced RIDD (ref. 52). Furthermore, IRE1α RNase activity was also linked to its phosphorylation status with key residues involved (that is, Ser724); where other phosphorylation sites mediate its inactivation in yeast[53,54]. However, the remaining identified phosphorylation sites have not yet been functionally tested[55]. Very recently, the missing link in IRE1α-mediated XBP1 mRNA splicing was uncovered. Whereas the *S. cerevisiae* ligase responsible for HAC1 mRNA (the yeast equivalent of XBP1) ligation was identified in 2008[56], the mammalian counterpart remained unknown. Four studies[57–60] have now uncovered the nature of the ligase as the tRNA ligase RtcB and demonstrated its physiological role in plasma cell differentiation[57]. Together, these discoveries shed light on the activation and signalling mechanisms of the three UPR sensors and on the biological networks that could be involved in their regulation.

The main biological consequences of UPR activation are the recovery of ER proteostasis (adaptive response) and the induction of a terminal UPR (apoptotic phase). One of the first responses to ER homeostasis imbalance involves global translational attenuation to reduce entry of newly synthesized proteins into the ER. This is in part achieved through PERK-mediated phosphorylation of eIF2α but also through RIDD of mRNAs encoding secretory proteins or ribosomal RNAs. A general mechanism leading to translation shutdown was also uncovered that results in massive detachment of translating ribosomes from the ER membrane upon ER stress[61]. Another safeguard set in place by the ER to alleviate stress involves an increased clearance of misfolded proteins. Although this has been shown to be controlled by ERAD and autophagy[62,63], a recent study demonstrated that misfolded GPI-anchored proteins can escape ER retention and translocate to the plasma membrane where they are then degraded by the lysosomal pathway[64]. Finally, in order to re-establish homeostasis within the ER lumen, ER stress also leads to the activation of amino acid metabolism and improvement of antioxidant responses, in addition to the reinforcement of folding and quality-control mechanisms[65]. These events reduce the misfolding burden on the ER thereby allowing the organelle to recover homeostasis.

Cell death control under ER stress

When the buffering capacity of the UPR proves inadequate to restore ER proteostasis, the pathway alternates its signalling towards a terminal UPR (ref. 66). Although the molecular details are still being solved and may differ depending on cell type, accumulating evidence suggests that a distinct program of pro-apoptotic signals activate the cell death machinery if ER stress cannot be remedied (Fig. 2)[12,67]. For example, although a temporary halt in protein translation due to eIF2α phosphorylation can be advantageous for cells under ER stress, a prolonged block in translation from sustained PERK activation is incompatible with survival. Under chronic ER stress, persistent PERK signalling upregulates the transcription factor CHOP (also known as GADD153), which inhibits expression of the anti-apoptotic BCL-2 to promote cell death[68,69]. Moreover, it has been shown that CHOP and ATF4 cooperate to transcriptionally activate targets that enhance protein synthesis, and contribute to cell death through reactive oxygen species (ROS) production and ATP depletion[70]. PERK activation may also increase expression of death receptor 5 (DR5) to trigger caspase-8 induced cell death[71].

As mentioned above, chronic ER stress causes IRE1α to transition from a homodimeric state into higher order oligomeric structures, which appears to be the critical step in switching to its apoptotic program[50]. Sustained RIDD activity might deplete protein-folding components to further worsen ER stress[46]. IRE1α also induces the activation of a number of pro-inflammatory and pro-death proteins. For example, the RNase activity of IRE1α decreases the levels of select microRNAs that normally suppress pro-apoptotic targets such as pro-oxidant protein TXNIP (thioredoxin-interacting protein) and caspase-2, leading to their upregulation[48,49]. Increased TXNIP then activates the inflammasome and caspase-1[48]. Finally, under sustained engagement, IRE1α assembles into an activation platform for apoptosis signal-regulating kinase 1 (ASK1) and its downstream target c-Jun NH_2-terminal kinase (JNK)[72,73].

Many of the pro-death signals emerging from UPR sensors ultimately regulate the canonical mitochondrial apoptotic pathway. This pathway is initiated when pro-apoptotic mitochondrial proteins, such as cytochrome *c*, are actively released into the cytoplasm to trigger the proteolytic activation of effector caspases such as caspase-3. Pro- and anti-apoptotic proteins of the BCL-2 family govern the intrinsic apoptotic pathway by regulating the permeability of the outer mitochondrial membrane[74]. This pathway is set in motion when cell injury is sensed

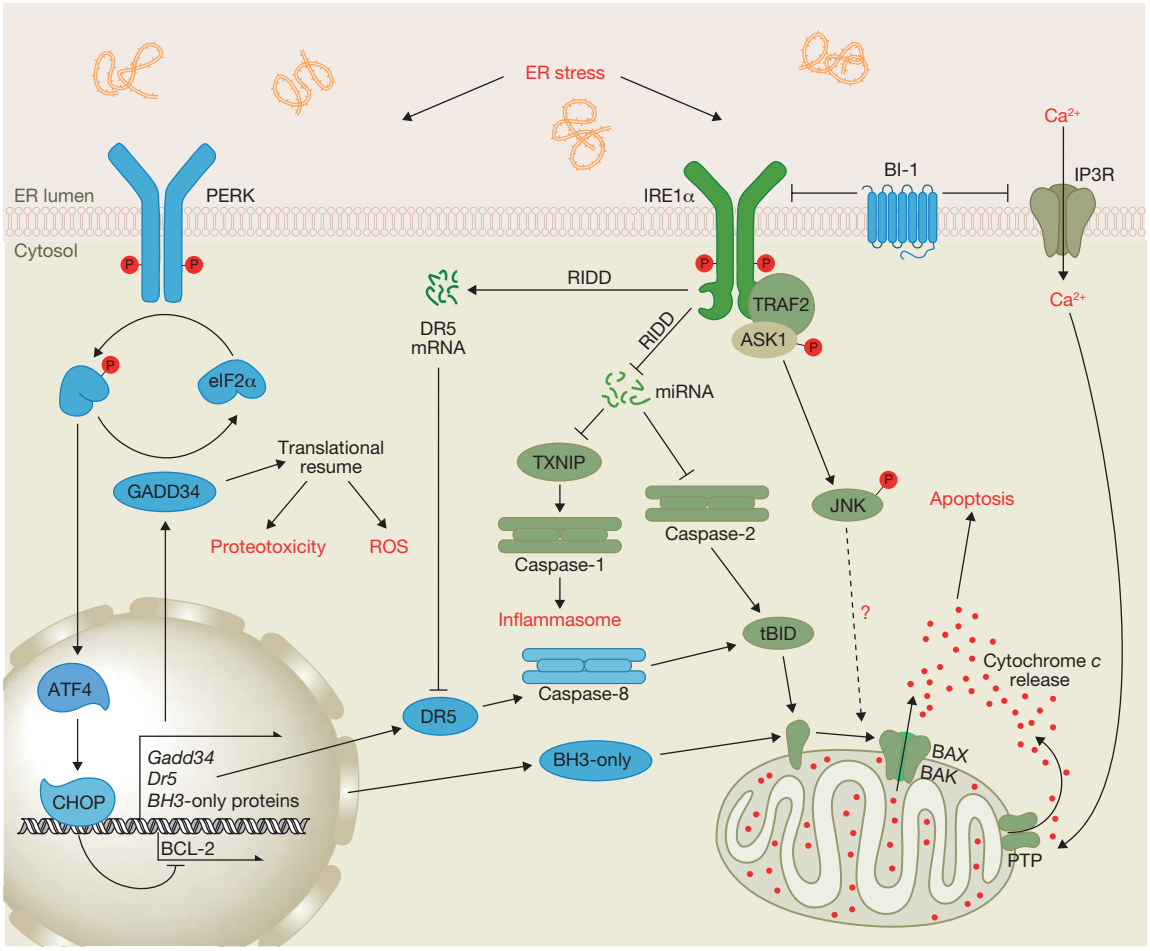

Figure 2 Under irremediable ER stress, the UPR actively promotes proteotoxicity, inflammatory responses and apoptosis. When exposed to chronically high levels of ER stress, PERK (pathway components in blue) and IRE1α (pathway components in green) both lead to cell dysfunction, activation of the inflammasome, and apoptosis (input and outcome depicted in red) through multiple signalling outputs. Among the ATF4 targets downstream of PERK (see Fig. 1) are also genes whose products are involved in the control of cell death (DR5 or BH3-only proteins) through the activation of signalling pathways from the plasma membrane or the mitochondria involving activation of BCL-2 family members BAX and BAK and release of apoptogenic factors such as cytochrome *c*. Similarly, IRE1α signals through the JNK and mRNA/miRNA degradation pathways to control cell survival. Interestingly, crosstalk between IRE1α and PERK signalling at the level of DR5, tightly control cell fate. ER calcium release also contributed to ER stress-mediated cell death, possibly involving IP3 receptors and the expression of TMBIM family members such as BI-1, which also inhibits IRE1α signalling. Overload of calcium leads to the activation of the permeability transition pore (PTP), triggering the release of cytochrome *c* and the activation of downstream caspases.

and leads to the transcriptional upregulation and/or post-translational activation of one or more BH3-only proteins, a collection of pro-death proteins all containing a short alpha helix known as the BCL-2 homology 3 (BH3) domain that is necessary for their killing activity[74,75]. Once activated, BH3-only proteins bind to and neutralize mitochondrial-protecting proteins (for example, BCL-2, BCL-X_L and MCL-1) and in some cases directly engage pro-apoptotic BAX and BAK proteins, causing their homo-oligomerization and resultant permeabilization of the outer mitochondrial membrane.

The terminal UPR can activate at least four distinct BH3-only proteins (BID, BIM, NOXA and PUMA) that then signal mitochondrial apoptosis[66]. As such, triple-knockout cells that are deficient in BIM, PUMA and BID are much more resistant to ER stress than cells deficient in any one BH3-only protein[76], a phenotype resembling a BAX and BAK double deficiency[77]. Each of these BH3-only proteins seems to be activated by ER stress in a unique way[66]. However, it remains unclear if these BH3-only proteins are simultaneously engaged by all forms of severe ER stress or if only a subset can be activated under specific insults. Another group of ancient cell death regulators, known as the TMBIM or the Bax-inhibitor 1 (BI-1) family[78], also has relevant activities against ER stress (that is, BI-1, also known as TMBIM6, and GRINA, also known as TMBIM3), possibly through the modulation of ER calcium release by the IP_3 receptors[79,80]. Although multiple mechanisms mediating ER stress-induced apoptosis are available, their individual impact on cell viability is partial, suggesting that combinatorial mechanisms or so called cell death networks control apoptosis when the ER is irreversibly damaged[67].

Cell fate decisions under ER stress

The UPR operates as a highly dynamic signalling network that integrates information about the intensity and duration of the stress stimuli and possibly the type of perturbation to the secretory pathway. Depending on the experimental system, distinct UPR branches operate independently

in terms of their activation pattern, signalling kinetics and downstream output signals. In this section, we discuss recent advances in defining how the UPR is fine-tuned, and its impact on determining the transition from adaptive to pro-apoptotic programs.

Analysis of the signalling kinetics of the three main UPR sensors has revealed that the temporal activation of each branch can vary depending on the nature of the pharmacological stressor used to perturb ER function[81,82]. Moreover, ATF6α can be selectively activated by the overload of the ER lumen with proteins[83], and its activity is also specifically modulated by its N-glycosylation state and the redox status of the ER (ref. 84,85). In addition, depending on the intensity of the stress, the signalling outputs of the UPR differ. Indeed low concentrations of ER stress agents engage all UPR signalling branches to initiate adaptive outputs, whereas high levels of ER stress results in apoptotic signals[86]. This is consistent with the observation that certain specialized secretory cells, such as pancreatic β-cells and B lymphocytes, are normally able to function under sustained levels of physiological ER stress without apoptosis induction[87].

The differential modulation of UPR signalling may underlay the mechanism behind the integration of global responses toward proteostasis recovery and the threshold to induce apoptosis. Despite the fact that the ER-sensing domains of IRE1α and PERK have similar primary structure, and are functionally interchangeable[88], the temporal pattern of their signalling drastically differs depending on the experimental setting. Under prolonged ER stress, XBP1 mRNA splicing is diminished, whereas PERK signalling is maintained leading to the expression of downstream pro-apoptotic components. This may also contribute to apoptosis by attenuating the survival effects of XBP1s, thus sensitizing cells to ER stress. Besides, PERK-induced CHOP expression could also upregulate GADD34, a component of the eIF2α phosphatase complex that reverts translational inhibition, triggering oxidative stress and proteotoxicity[69,89]. In other experimental settings, the sustained activation of IRE1α occurs under high ER stress to trigger cell death[43,46,50]. Using single cell imaging, a recent report also suggested that the relative kinetics of PERK and IRE1α signalling determines the induction of apoptosis rather than a switch between both branches[90]. These observations emphasize the highly dynamic and complex nature of the UPR regulatory network.

Given that the ER sensing domains of IRE1α and PERK are similar, the temporal behaviour of UPR signalling may be in part mediated by intrinsic conformational changes of the sensors, post-translational modifications and/or the binding to specific positive and negative regulators (Fig. 3). Most of the studies addressing the molecular basis of fine-tuning UPR activity have been performed with IRE1α. The concept of the 'UPRosome' envisions IRE1α as a scaffold where many components assemble to selectively regulate its activity (amplitude and kinetics) and the control of specific downstream signals (Fig. 3a)[91]. IRE1α activation and inactivation are instigated by a direct binding of several factors to the UPRosome that modulate the amplitude of UPR responses without affecting PERK (ref. 92). Interestingly, many regulators of IRE1α have relevant roles in apoptosis[93], including members of the BCL-2 family such as BAX, BAK (ref. 94), some BH3-only proteins[95], components of the MAP kinase pathway[96–98], and its negative regulator BI-1[99,100]. Although the list of IRE1-binding partners is increasing and a few screens have been recently reported, systematic interactome studies are still missing. Here we highlight recent discoveries of interacting proteins that modulate the sustained signalling of IRE1α under prolonged ER stress. N-MYC Interactor (NMI) protein is an IRE1α-binding partner identified through an interactome screen, which selectively enhances JNK activation and apoptosis, but does not affect XBP1 mRNA splicing[101]. Another proteomic screen also found non-muscle myosin heavy chain IIB (NMIIB), and possibly actin cytoskeleton, as a specific regulator of IRE1α clustering (Fig. 3a), without affecting its dimerization and phosphorylation process[102]; a mechanism also validated in yeast[103]. Furthermore, specific protein complexes may be assembled at the level of the IRE1α ER luminal domain. BiP has been known for more than a decade[13] to operate as a negative regulator of IRE1α. The disulphide isomerase PDIA6 was recently shown to control activation and/or inactivation of IRE1α signalling through a direct binding[104,105]. Hence, IRE1α signalling is fine-tuned by the assembly of distinct signalling complexes at the level of its cytosolic and ER-luminal regions.

Recent discoveries also indicate that PERK and ATF6 are regulated through the binding of specific factors (Fig. 3a,b). For example, the kinase activity of PERK is selectively reduced by the binding of p58IPK (ref. 106), a regulation antagonized by a cytosolic variant of BiP known as GRP78va (ref. 107). An interactome analysis identified transducin β-like 2 (TBL2) as a protein that associates with phosphorylated PERK under stress conditions, determining optimal signalling to drive ATF4 expression and stress mitigation[108]. The small GTPase Rheb also binds and activates PERK, repressing protein translation[109]. Finally, PDIA6 also binds to PERK and negatively modulates the decay of its signalling[104]. In the case of ATF6α, a few direct regulators have been reported such as Wolfram syndrome 1 (WFS1), which mediates the degradation of ATF6 by the proteasome[110]. Since the reduction of intra- and inter-luminal disulphide bonds in ATF6α modulate its translocation to the Golgi, a cell-based RNAi screen was performed to target most PDIs and ER oxidoreductases[111]. This study identified PDIA5 as a selective and critical regulator of ATF6α activation. Overall, these novel studies highlight the concept that all UPR branches are modulated by protein–protein interactions through the assembly of dynamic complexes or UPRosomes that control the amplitude and temporal behaviour of their signalling.

Although less explored, the UPR is also fine-tuned by post-translational modifications at the level of ER sensors and transcription factors. In the context of glucose metabolism, IRE1α function is directly instigated through the phosphorylation of Ser724 by PKA (ref. 112). IRE1α phosphorylation is attenuated by different phosphatases, including PP2A (ref. 113) and PPM1 (refs 114,115), which were identified through proteomic screens. Protein tyrosine phosphatase-1B (PTP-1B) also has a selective effect on bursting IRE1α signalling[116]. Moreover, ADP-ribosylation of PERK and IRE1α enhances their activities (Fig. 3a,b)[117], and IRE1α ubiquitylation is mediated by the E3 ligase CHIP, which increases JNK signalling without affecting XBP1 mRNA splicing[118]. p38MAPK phosphorylates ATF6α (ref. 119) and XBP1s (ref. 120), having a positive effect on gene expression. XBP1s activity is also modulated by acetylation and sumoylation[121,122]. Moreover, the association of ATF6f, ATF4 and XBP1s with other transcription factors determines the establishment of stimuli- and tissue-specific transcriptional patterns[92,123]. In agreement with this concept, genome-wide transcriptional profiling under ER stress indicated that the genetic background highly influences the pattern of gene regulation[124,125]. Finally, non-sense mediated RNA decay also determines the threshold of stress necessary to activate the UPR (refs 126,127), in addition to adjusting the amplitude of downstream responses and the

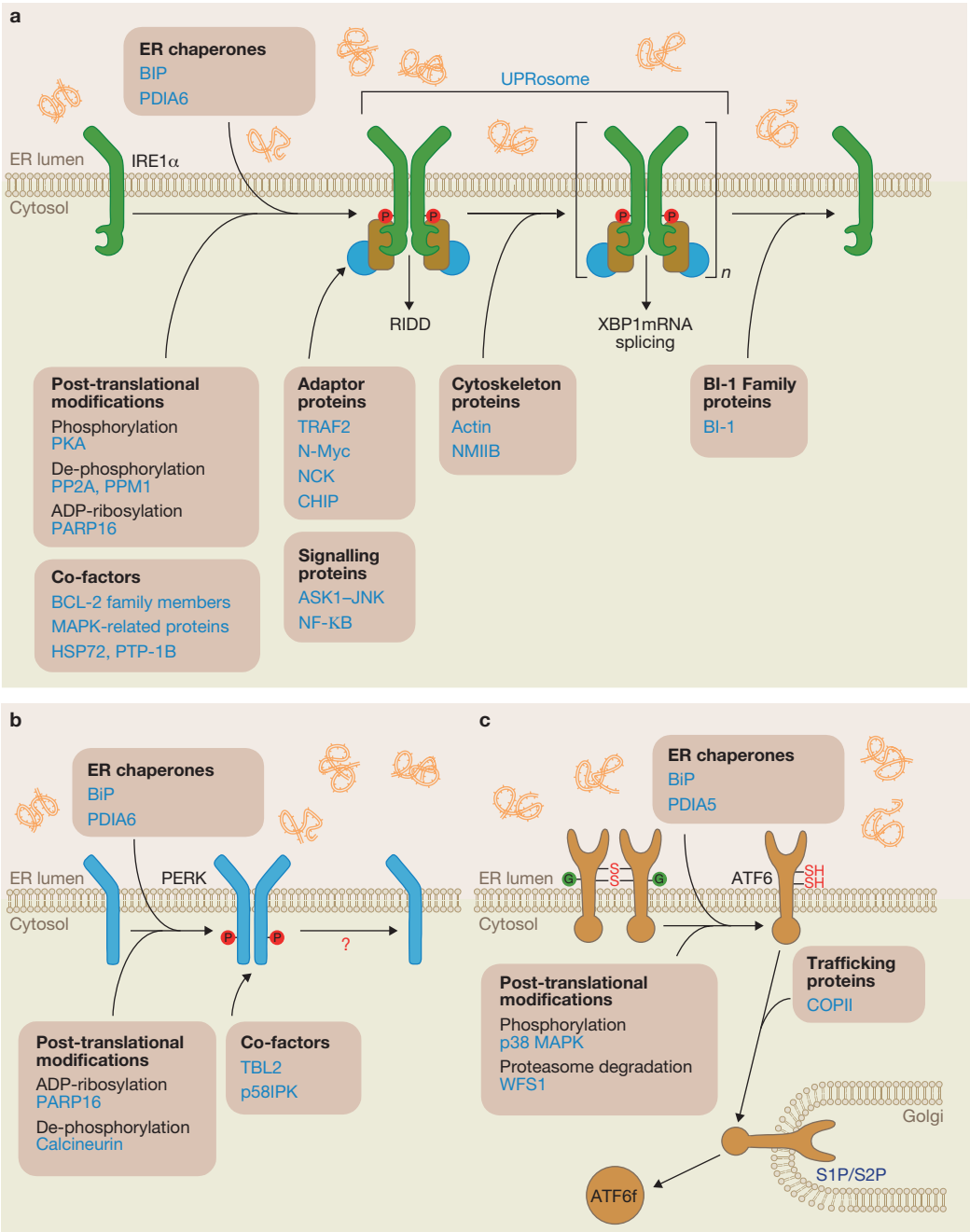

Figure 3 The UPRosome: fine-tuning ER stress signalling through protein–protein interactions and/or post-translational modifications. The activity of UPR stress sensors is regulated through the binding of co-factors and post-translational modifications that modulate the amplitude of their downstream signals and the kinetics of activation and attenuation. The assembly of distinct UPRosomes may also mediate the crosstalk of the UPR with other signalling pathways, and the control of novel stress-independent functions. (**a**) Fine-tuning IRE1α signalling through the dissociation of ER chaperones in the activation phase, recruitment of the UPRosome that controls signalling outputs and inactivation phase. (**b**) Fine-tuning PERK activation through the dissociation of BiP in the activation phase and the control of phosphorylation and ADP-ribosylation. (**c**) ATF6α activation depends on its N-glycosylation and redox status in addition to the dissociation from BiP to allow egress from the ER and proteolytic cleavage in the Golgi apparatus by the S1P and S2P proteases. In addition, ATF6α signalling output is controlled at the level of protein stability.

termination phase by controlling the mRNA stability of UPR components[128]. These observations highlight the dynamic crosstalk between mRNA metabolism and the proteostasis network. These examples illustrate that the strength and temporal activation/inactivation of each UPR signalling arm is independently regulated at different stages depending on the stress condition and the tissue/cell type affected.

Although our understanding of how the UPR network is regulated is constantly growing, the mechanisms underlying the integration of the stress intensity and its duration are not well described. It is becoming clear that the assembly of UPRosome regulatory complexes determines the threshold of activation of UPR sensors and modulates the final outcome of the pathway. One of the major limitations in the field is the fact

that most studies rely on the use of acute stressors that irreversibly damage the ER, leading to apoptosis. These 'non-physiological' conditions normally trigger the upregulation of pro-survival and pro-apoptotic components with virtually identical kinetics. Importantly, an approach was recently developed to model 'resolvable' ER stress, leading to full activation of the adaptive UPR in the absence of apoptosis features[129]. Using this approach, a signalling crosstalk was discovered that mediates adaptation to ER stress involving oestrogen signalling[129]. We believe these technological advances will enable the examination of cell fate mechanisms under ER stress in a tractable system.

Novel functions of the UPR

Secreted factors can control extracellular proteostasis. There is a continuum between the ER lumen and the extracellular space; and stressed cells have developed ways to control their microenvironment by secreting key enzymes. The most recent example of this mechanism is illustrated by the secretion of ERdj3, which extracellularly binds misfolded proteins, inhibits their aggregation and attenuates proteotoxicity of disease-associated prion protein[130]. Similarly, enforced expression of XBP1s or ATF6α in a stress-independent manner reduces extracellular aggregation of amyloidogenic immunoglobulin variants[131]. Other chaperones of the GRP and PDI family are also secreted through a regulatory mechanism dependent on ER stress[132]. This phenomenon is also observed with redox proteins such as QSOX1, which controls the structure of the extracellular matrix[133] most likely in conjunction with secreted PDI family members such as ERp57 (ref. 134).

Stress-independent functions of the UPR have been identified. The crosstalk between plasma membrane signalling and the ER is illustrated by cell surface receptors and UPR components (Fig. 4a). In macrophages, optimal secretion of pro-inflammatory cytokines by Toll-like receptors (TLR) is mediated by XBP1, where TLR stimulation engages IRE1α in the absence of global ER stress markers, possibly mediated by NADPH oxidase 2 signalling[135]. Moreover, TLR signalling may even signal to repress ATF4–CHOP expression[136,137]. Similarly, the well-known function of XBP1s in plasma cell differentiation[138] may occur in a stress-independent manner, initiated by the signalling of B cell receptor to control cell differentiation programs[139]. In agreement with this concept, XBP1 modulates the activity of MIST1, a master regulator of cell differentiation in different cell types[140]. IRE1α has been also proposed to monitor fluctuation in glucose levels in the absence of ER stress, mediated by its phosphorylation, occuring independently of the release of BiP from the luminal domain[113,141]. Similarly, CD40 signalling increases XBP1 mRNA splicing and thus protects the secretory pathway of hepatocytes from ER stress induced by either tunicamycin or oleic acid[142]. In endothelial cells, VEGF activates all UPR mediators via signalling through a PLCγ-mediated crosstalk with mTORC1 in an ER stress-independent manner, contributing to endothelial cell survival and angiogenesis[143]. Thus, UPR signalling modules may have important cellular functions unrelated to ER stress.

Functional and physical connections between the ER and mitochondria have long been described in the literature, especially in the field of apoptosis and in mitochondrial dynamics. PERK is located at mitochondrial associated membranes (MAMs), where it is required to maintain the ER–mitochondria juxtapositions and the production of ROS to engage mitochondrial-dependent apoptosis[144,145] (Fig. 4b). These observations were associated with a physical interaction between PERK and the

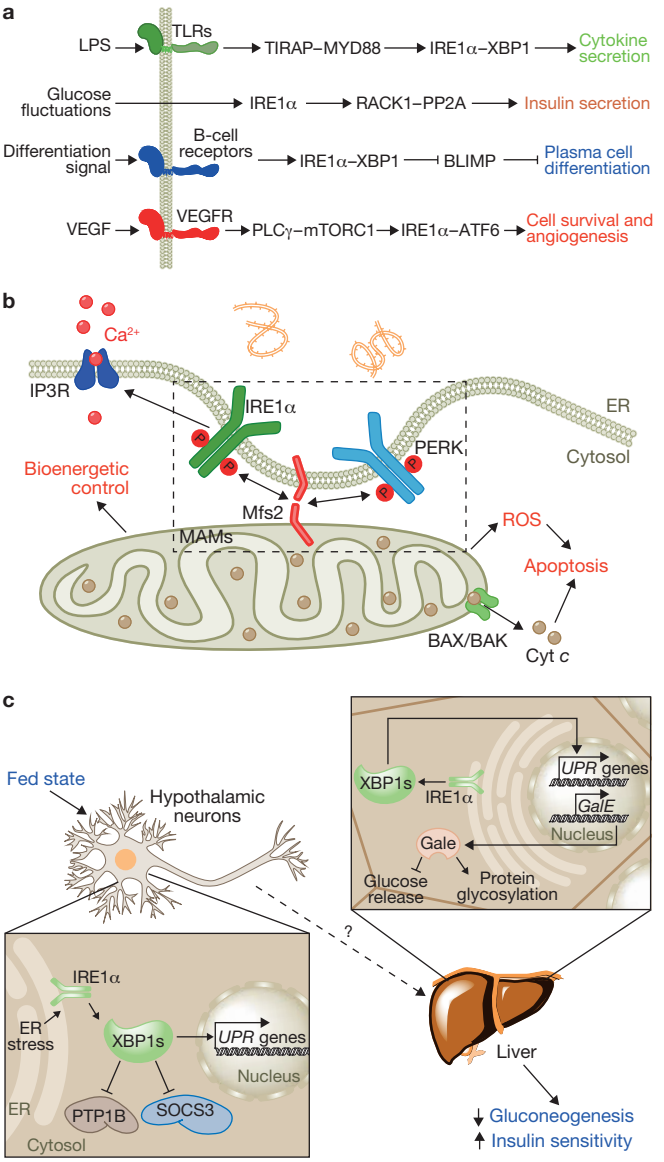

Figure 4 ER stress-independent functions of the UPR. (**a**) Signalling (LPS, glucose, differentiation, VEGF)-dependent and protein misfolding-independent activation of the canonical UPR sensors. (**b**) Control of mitochondrial function by UPR signalling at the ER-mitochondrion interface. (**c**) Cell-nonautonomous UPR activation. ER stress is triggered in neurons that subsequently prompt the activation of a subset of UPR signals in the non-stressed liver through yet uncharacterized mediators.

ER tethering protein mitofusin 2 (ref. 145). Interestingly, mitofusin 2 deficiency leads to chronic ER stress[146]. IRE1α has been also detected in MAMs[147], and might contribute to apoptosis by modulating calcium transfer and ROS production by the mitochondria[148]. Obesity leads to a marked reorganization of MAMs in the liver, resulting in mitochondrial calcium overload and compromised mitochondrial oxidative capacity[149]. Whether the localization of UPR stress sensors at MAMs affects mitochondrial bioenergetics and its dynamics remains an open question.

Classically, the mechanisms controlling responses to altered proteostasis are considered 'cell intrinsic' (also termed cell-autonomous). Recent studies in the field have provided evidence for regulation of

stress responses at a distance, largely mediated by the nervous system, on a cell-nonautonomous manner[150]. One of the first examples for cell-nonautonomous control of the UPR came from aging studies in *Caenorhabditis elegans*. Enforced expression of XBP1s in neurons increases life span of this model organism[151]. Remarkably, neuronal XBP1s is able to promote IRE1α activation and XBP1 mRNA splicing in peripheral tissues, and this stress signalling propagation was crucial to protect against aging[151]. An analogous cell-nonautonomous regulatory circuit was only recently uncovered in mammals where hypothalamic XBP1s expression modulates global metabolism through a cell-nonautonomous propagation of UPR signals to the liver[152] (Fig. 4c). In all these studies, whether the activation of the UPR in the distant cell is ER-stress-independent remains to be clarified. The activation of a cell-nonautonomous UPR is emerging as an evolutionary conserved mechanism to prevent uncontrolled protein misfolding on an organismal level. Thus, the UPR may be also fine-tuned at the level of complex tissues and even the whole organism.

Concluding remarks

The UPR is a conserved signal transduction pathway activated when cells fail to keep up with the protein folding demands on the ER. In response to mild ER stress, the UPR promotes adaptive outputs that reduce unfolded protein load and improve the ability of the secretory pathway to restore proteostasis. However, under irreversible ER stress, the UPR assembles into an alternate platform that engages a complex network of signals to hasten cell demise. Cell injury due to ongoing ER stress has emerged as a central contributor to the pathophysiology of a wide range of common human diseases and aging. Recent advances in our knowledge of how the UPR shifts from life-to-death signalling, and the development of small molecule inhibitors of the UPR (ref. 153), are the stepping stones for new strategies to combat these ER stress-associated diseases. Despite the immense progress made over the past decade into the biological relevance of the UPR, many questions remain open. The next few years promise to shed much needed light on the coordination and specific contribution of the three arms of the UPR in health and disease; what role each plays in the absence of ER stress; and how they are regulated at the whole-organism level. Many novel stress-independent functions of UPR signalling modules are also emerging as relevant contributors to cell physiology and disease, which may involve allosteric and post-translational modifications to stress sensors through the assembly of distinct UPRosome complexes. It is becoming clear that this field is gaining complexity each year and that the UPR can no longer be viewed as a linear pathway, as multiple modulatory steps and dynamic crosstalk operate to integrate the UPR with the global proteostasis network and other relevant signalling pathways.

ACKNOWLEDGEMENTS

We thank Hery Urra and Victor Hugo Cornejo for figure design. Funded by ECOS-CONICYT C13S02 (C.H., E.C.), Ring Initiative-ACT1109, FONDEF-D11I1007, Millennium Institute-P09-015-F, the Frick Foundation, FONDECYT no. 1140549, Michael J. Fox Foundation for Parkinson Research, COPEC-UC Foundation, CONICYT grant USA2013-0003 (C.H.), the Institut National du Cancer (PLBio and ICGC; INCa) and La Ligue Nationale Contre le Cancer (LARGE) (E.C.); NIH: RO1CA136577 and R01-DK095306, American Cancer Society Research Scholar Award, Harrington Discovery Institute Scholar-Innovator Award, Michael J. Fox Foundation for Parkinson's Research, Caring for Carcinoid Foundation and American Association for Cancer Research (S.A.O.).

ADDITIONAL INFORMATION

Reprints and permissions information is available online at www.nature.com/reprints. Correspondence should be addressed to C.H.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Balch, W. E., Morimoto, R. I., Dillin, A. & Kelly, J. W. Adapting proteostasis for disease intervention. *Science* **319,** 916–919 (2008).
2. Schubert, U. *et al.* Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* **404,** 770–774 (2000).
3. Seino, S., Shibasaki, T. & Minami, K. Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *J. Clin. Invest.* **121,** 2118–2125 (2011).
4. Wang, S. & Kaufman, R. J. The impact of the unfolded protein response on human disease. *J. Cell Biol.* **197,** 857–867 (2012).
5. Kozutsumi, Y., Segal, M., Normington, K., Gething, M. J. & Sambrook, J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature* **332,** 462–464 (1988).
6. Mori, K., Ma, W., Gething, M. J. & Sambrook, J. A transmembrane protein with a cdc2+CDC28-related kinase activity is required for signalling from the ER to the nucleus. *Cell* **74,** 743–756 (1993).
7. Cox, J. S. & Walter, P. A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell* **87,** 391–404 (1996).
8. Tirasophon, W., Welihinda, A. A. & Kaufman, R. J. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev.* **12,** 1812–1824 (1998).
9. Harding, H. P., Zhang, Y. & Ron, D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* **397,** 271–274 (1999).
10. Haze, K., Yoshida, H., Yanagi, H., Yura, T. & Mori, K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol. Biol. Cell* **10,** 3787–3799 (1999).
11. Ron, D. & Walter, P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **8,** 519–529 (2007).
12. Tabas, I. & Ron, D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat. Cell Biol.* **13,** 184–190 (2011).
13. Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P. & Ron, D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* **2,** 326–332 (2000).
14. Shen, J., Chen, X., Hendershot, L. & Prywes, R. ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. *Dev. Cell* **3**, 99–111 (2002).
15. Carrara, M., Prischi, F., Nowak, P. R., Kopp, M. C. & Ali, M. M. Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein C_H1 to initiate ER stress signaling. *eLife* **4,** e03522 (2015).
16. Credle, J. J., Finer-Moore, J. S., Papa, F. R., Stroud, R. M. & Walter, P. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc. Natl Acad. Sci. USA* **102,** 18773–18784 (2005).
17. Gardner, B. M. & Walter, P. Unfolded Proteins Are Ire1-Activating Ligands that Directly Induce the Unfolded Protein Response. *Science* **333,** 1891–1894 (2011).
18. Oikawa, D., Kitamura, A., Kinjo, M. & Iwawaki, T. Direct association of unfolded proteins with mammalian ER stress sensor, IRE1β. *PLoS ONE* **7,** e51290 (2012).
19. Kimata, Y. & Kohno, K. Endoplasmic reticulum stress-sensing mechanisms in yeast and mammalian cells. *Curr. Opin. Cell Biol.* **23,** 135–142 (2011).
20. Oikawa, D., Kimata, Y., Kohno, K. & Iwawaki, T. Activation of mammalian IRE1α upon ER stress depends on dissociation of BiP rather than on direct interaction with unfolded proteins. *Exp. Cell Res* **315**, 2496–2504 (2009).
21. Zhou, J. *et al.* The crystal structure of human IRE1 luminal domain reveals a conserved dimerization interface required for activation of the unfolded protein response. *Proc. Natl Acad. Sci. USA* **103,** 14343–14348 (2006).
22. Scheuner, D. *et al.* Translational control is required for the unfolded protein response and *in vivo* glucose homeostasis. *Mol. Cell* **7,** 1165–1176 (2001).
23. Harding, H. P. *et al.* Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* **6,** 1099–1108 (2000).
24. Harding, H. P. *et al.* An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell* **11,** 619–633 (2003).
25. Ye, J. & Koumenis, C. ATF4, an ER stress and hypoxia-inducible transcription factor and its potential role in hypoxia tolerance and tumorigenesis. *Curr. Mol. Med.* **9,** 411–416 (2009).
26. Del Vecchio, C. A. *et al.* De-differentiation confers multidrug resistance via noncanonical PERK-Nrf2 signaling. *PLoS Biol.* **12,** e1001945 (2014).
27. Cullinan, S. B. & Diehl, J. A. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J. Biol. Chem.* **279,** 20108–20117 (2004).
28. Cullinan, S. B. *et al.* Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol. Cell Biol.* **23,** 7198–7209 (2003).
29. Asada, R., Kanemoto, S., Kondo, S., Saito, A. & Imaizumi, K. The signalling from endoplasmic reticulum-resident bZIP transcription factors involved in diverse cellular physiology. *J. Biochem.* **149,** 507–518 (2011).
30. Yamamoto, K. *et al.* Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1. *Dev. Cell* **13,** 365–376 (2007).
31. Shen, X. *et al.* Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development. *Cell* **107,** 893–903 (2001).
32. Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. & Mori, K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107,** 881–891 (2001).

33. Lee, K. *et al.* IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev* **16,** 452–466 (2002).
34. Calfon, M. *et al.* IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415,** 92–96 (2002).
35. Hetz, C., Martinon, F., Rodriguez, D. & Glimcher, L. H. The unfolded protein response: integrating stress signals through the stress sensor IRE1α. *Physiol. Rev.* **91,** 1219–1243 (2011).
36. Acosta-Alvear, D. *et al.* XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. *Mol. Cell* **27,** 53–66 (2007).
37. Lee, A. H., Iwakoshi, N. N. & Glimcher, L. H. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol. Cell Biol.* **23,** 7448–7459 (2003).
38. Shoulders, M. D. *et al.* Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER proteostasis environments. *Cell Rep.* **3,** 1279–1292 (2013).
39. Yanagitani, K. *et al.* Cotranslational targeting of XBP1 protein to the membrane promotes cytoplasmic splicing of its own mRNA. *Mol. Cell* **34,** 191–200 (2009).
40. Yanagitani, K., Kimata, Y., Kadokura, H. & Kohno, K. Translational pausing ensures membrane targeting and cytoplasmic splicing of *XBP1u* mRNA. *Science* **331,** 586–589 (2011).
41. Yoshida, H., Oku, M., Suzuki, M. & Mori, K. pXBP1(U) encoded in XBP1 pre-mRNA negatively regulates unfolded protein response activator pXBP1(S) in mammalian ER stress response. *J. Cell Biol.* **172,** 565–575 (2006).
42. Chen, C. Y. *et al.* Signal peptide peptidase functions in ERAD to cleave the unfolded protein response regulator XBP1u. *EMBO J.* **33,** 2492–2506 (2014).
43. Maurel, M., Chevet, E., Tavernier, J. & Gerlo, S. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* **39,** 245–254 (2014).
44. Hollien, J. & Weissman, J. S. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* **313,** 104–107 (2006).
45. Hollien, J. *et al.* Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J. Cell Biol.* **186,** 323–331 (2009).
46. Han, D. *et al.* IRE1α kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell* **138,** 562–575 (2009).
47. Iwawaki, T. *et al.* Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. *Nat. Cell Biol.* **3,** 158–164 (2001).
48. Lerner, A. G. *et al.* IRE1α induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab.* **16,** 250–264 (2012).
49. Upton, J. P. *et al.* IRE1α cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. *Science* **338,** 818–822 (2012).
50. Ghosh, R. *et al.* Allosteric inhibition of the IRE1α RNase preserves cell viability and function during endoplasmic reticulum stress. *Cell* **158,** 534–548 (2014).
51. Tam, A. B., Koong, A. C. & Niwa, M. Ire1 Has Distinct Catalytic Mechanisms for XBP1/HAC1 Splicing and RIDD. *Cell Rep.* **9,** 1–9 (2014).
52. Bouchecareilh, M., Higa, A., Fribourg, S., Moenner, M. & Chevet, E. Peptides derived from the bifunctional kinase/RNase enzyme IRE1α modulate IRE1α activity and protect cells from endoplasmic reticulum stress. *FASEB J.* **25,** 3115–3129 (2011).
53. Rubio, C. *et al.* Homeostatic adaptation to endoplasmic reticulum stress depends on Ire1 kinase activity. *J. Cell Biol.* **193,** 171–184 (2011).
54. Chawla, A., Chakrabarti, S., Ghosh, G. & Niwa, M. Attenuation of yeast UPR is essential for survival and is mediated by IRE1 kinase. *J. Cell Biol.* **193,** 41–50 (2011).
55. Prischi, F., Nowak, P. R., Carrara, M. & Ali, M. M. Phosphoregulation of Ire1 RNase splicing activity. *Nat. Commun.* **5,** 3554 (2014).
56. Harding, H. P. *et al.* An intact unfolded protein response in Trpt1 knockout mice reveals phylogenic divergence in pathways for RNA ligation. *RNA* **14,** 225–232 (2008).
57. Jurkin, J. *et al.* The mammalian tRNA ligase complex mediates splicing of XBP1 mRNA and controls antibody secretion in plasma cells. *EMBO J.* **33,** 2922–2936 (2014).
58. Kosmaczewski, S. G. *et al.* The RtcB RNA ligase is an essential component of the metazoan unfolded protein response. *EMBO Rep.* **15,** 1278–1285 (2014).
59. Lu, Y., Liang, F. X. & Wang, X. A synthetic biology approach identifies the mammalian UPR RNA ligase RtcB. *Mol. Cell* **55,** 758–770 (2014).
60. Ray, A., Zhang, S., Rentas, C., Caldwell, K. A. & Caldwell, G. A. RTCB-1 mediates neuroprotection via XBP-1 mRNA splicing in the unfolded protein response pathway. *J Neurosci.* **34,** 16076–16085 (2014).
61. Reid, D. W., Chen, Q., Tay, A. S., Shenolikar, S. & Nicchitta, C. V. The unfolded protein response triggers selective mRNA release from the endoplasmic reticulum. *Cell* **158,** 1362–1374 (2014).
62. Ogata, M. *et al.* Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol. Cell. Biol.* **26,** 9220–9231 (2006).
63. Senft D & Ronai Z. A. UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem. Sci.* **40,** 141–8 (2015).
64. Satpute-Krishnan, P. *et al.* ER stress-induced clearance of misfolded GPI-anchored proteins via the secretory pathway. *Cell* **158,** 522–533 (2014).
65. Schroder, M. & Kaufman, R. J. The mammalian unfolded protein response. *Annu. Rev. Biochem.* **74,** 739–789 (2005).
66. Shore, G. C., Papa, F. R. & Oakes, S. A. Signaling cell death from the endoplasmic reticulum stress response. *Curr. Opin. Cell Biol.* **23,** 143–149 (2011).
67. Urra, H., Dufey, E., Lisbona, F., Rojas-Rivera, D. & Hetz, C. When ER stress reaches a dead end. *Biochim. Biophys. Acta* **1833,** 3507–3517 (2013).
68. McCullough, K. D., Martindale, J. L., Klotz, L. O., Aw, T. Y. & Holbrook, N. J. Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Mol. Cell Biol.* **21,** 1249–1259 (2001).
69. Marciniak, S. J. *et al.* CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev.* **18,** 3066–3077 (2004).
70. Han, J. *et al.* ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat. Cell Biol.* **15,** 481–490 (2013).
71. Lu, M. *et al.* Cell death. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis. *Science* **345,** 98–101 (2014).
72. Urano, F. *et al.* Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* **287,** 664–666 (2000).
73. Nishitoh, H. *et al.* ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev.* **16,** 1345–1355 (2002).
74. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116,** 205–219 (2004).
75. Tait, S. W. & Green, D. R. Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat. Rev. Mol. Cell Biol.* **11,** 621–632 (2010).
76. Ren, D. *et al.* BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program. *Science* **330**, 1390–1393 (2010).
77. Wei, M. C. *et al.* Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* **292,** 727–730 (2001).
78. Rojas-Rivera, D. & Hetz, C. TMBIM protein family: ancestral regulators of cell death. *Oncogene* **34,** 269–280 (2015).
79. Rojas-Rivera, D. *et al.* TMBIM3/GRINA is a novel unfolded protein response (UPR) target gene that controls apoptosis through the modulation of ER calcium homeostasis. *Cell Death Differ.* **19,** 1013–1026 (2014).
80. Chae, H. J. *et al.* BI-1 regulates an apoptosis pathway linked to endoplasmic reticulum stress. *Mol. Cell* **15,** 355–366 (2004).
81. Yoshida, H. *et al.* A time-dependent phase shift in the mammalian unfolded protein response. *Dev. Cell* **4,** 265–271 (2003).
82. DuRose, J. B., Tam, A. B. & Niwa, M. Intrinsic capacities of molecular sensors of the unfolded protein response to sense alternate forms of endoplasmic reticulum stress. *Mol. Biol. Cell* **17,** 3095–3107 (2006).
83. Maiuolo, J., Bulotta, S., Verderio, C., Benfante, R. & Borgese, N. Selective activation of the transcription factor ATF6 mediates endoplasmic reticulum proliferation triggered by a membrane protein. *Proc. Natl Acad. Sci. USA* **108,** 7832–7837 (2011).
84. Hong, M. *et al.* Underglycosylation of ATF6 as a novel sensing mechanism for activation of the unfolded protein response. *J. Biol. Chem.* **279,** 11354–11363 (2004).
85. Nadanaka, S., Okada, T., Yoshida, H. & Mori, K. Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress. *Mol. Cell Biol.* **27,** 1027–1043 (2007).
86. Rutkowski, D. T. *et al.* Adaptation to ER stress is mediated by differential stabilities of pro-survival and pro-apoptotic mRNAs and proteins. *PLoS Biol.* **4,** e374 (2006).
87. Cornejo, V. H., Pihan, P., Vidal, R. L. & Hetz, C. Role of the unfolded protein response in organ physiology: lessons from mouse models. *IUBMB Life* **65,** 962–975 (2013).
88. Liu, C. Y., Schroder, M. & Kaufman, R. J. Ligand-independent dimerization activates the stress response kinases IRE1 and PERK in the lumen of the endoplasmic reticulum. *J. Biol. Chem.* **275,** 24881–24885 (2000).
89. Novoa, I., Zeng, H., Harding, H. P. & Ron, D. Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2α. *J. Cell Biol.* **153,** 1011–1022 (2001).
90. Walter, F., Schmid, J., Dussmann, H., Concannon, C. G. & Prehn, J. H. Imaging of single cell responses to ER stress indicates that the relative dynamics of IRE1/XBP1 and PERK/ATF4 signalling rather than a switch between signalling branches determine cell survival. *Cell Death Differ.* http://dx.doi.org/10.1038/cdd.2014.241 (2015).
91. Hetz, C. & Glimcher, L. H. Fine-tuning of the unfolded protein response: Assembling the IRE1α interactome. *Mol. Cell* **35,** 551–561 (2009).
92. Hetz, C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.* **13,** 89–102 (2012).
93. Woehlbier, U. & Hetz, C. Modulating stress responses by the UPRosome: a matter of life and death. *Trends Biochem. Sci.* **36,** 329–337 (2011).
94. Hetz, C. *et al.* Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1α. *Science* **312,** 572–576 (2006).
95. Rodriguez, D.a. *et al.* BH3-only proteins are part of a regulatory network that control the sustained signalling of the unfolded protein response sensor IRE1α. *EMBO J.* **31,** 2322–2335 (2012).
96. Luo, D. *et al.* AIP1 is critical in transducing IRE1-mediated endoplasmic reticulum stress response. *J. Biol. Chem.* **283,** 11905–11912 (2008).
97. Yoneda, T. *et al.* Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J. Biol. Chem.* **276,** 13935–13940 (2001).
98. Oono, K. *et al.* JAB1 participates in unfolded protein responses by association and dissociation with IRE1. *Neurochem. Int.* **45,** 765–772 (2004).
99. Lisbona, F. *et al.* BAX inhibitor-1 is a negative regulator of the ER stress sensor IRE1α. *Mol Cell* **33,** 679–691 (2009).
100. Castillo, K. *et al.* BAX inhibitor-1 regulates autophagy by controlling the IRE1α branch of the unfolded protein response. *EMBO J.* **30,** 4465–4478 (2011).
101. Brozzi, F. *et al.* A combined "omics" approach identifies N-Myc interactor as a novel cytokine-induced regulator of IRE1 protein and c-Jun N-terminal kinase in pancreatic beta cells. *J. Biol. Chem.* **289,** 20677–20693 (2014).
102. He, Y. *et al.* Nonmuscle myosin IIB links cytoskeleton to IRE1α signaling during ER stress. *Dev. Cell* **23,** 1141–1152 (2012).
103. Ishiwata-Kimata, Y., Yamamoto, Y. H., Takizawa, K., Kohno, K. & Kimata, Y. F-actin and a type-II myosin are required for efficient clustering of the ER stress sensor Ire1. *Cell Struct. Funct.* **38,** 135–143 (2013).
104. Eletto, D., Dersh, D., Gidalevitz, T. & Argon, Y. Protein disulfide isomerase A6 controls the decay of IRE1α signaling via disulfide-dependent association. *Mol. Cell* **53,** 562–576 (2014).

105. Groenendyk, J. *et al.* Interplay between the oxidoreductase PDIA6 and microRNA-322 controls the response to disrupted endoplasmic reticulum calcium homeostasis. *Sci. Signal.* **7,** ra54 (2014).
106. Yan, W. *et al.* Control of PERK eIF2α kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58^{IPK}. *Proc. Natl Acad. Sci. USA* **99,** 15920–15925 (2002).
107. Ni, M., Zhou, H., Wey, S., Baumeister, P. & Lee, A. S. Regulation of PERK signaling and leukemic cell survival by a novel cytosolic isoform of the UPR regulator GRP78/BiP. *PLoS ONE* **4,** e6868 (2009).
108. Tsukumo, Y. *et al.* TBL2 is a novel PERK-binding protein that modulates stress-signaling and cell survival during endoplasmic reticulum stress. *PLoS ONE* **9,** e112761 (2014).
109. Tyagi, R. *et al.* Rheb inhibits protein synthesis by activating the PERK-eIF2α signaling cascade. *Cell Rep.* **10,** 684–693 (2015).
110. Fonseca, S. G. *et al.* Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. *J. Clin. Invest.* **120,** 744–755 (2010).
111. Higa, A. *et al.* Endoplasmic reticulum stress-activated transcription factor ATF6α requires the disulfide isomerase PDIA5 to modulate chemoresistance. *Mol. Cell Biol.* **34,** 1839–1849 (2014).
112. Mao, T. *et al.* PKA phosphorylation couples hepatic inositol-requiring enzyme 1α to glucagon signaling in glucose metabolism. *Proc. Natl Acad. Sci. USA* **108,** 15852–15857 (2011).
113. Qiu, Y. *et al.* A crucial role for RACK1 in the regulation of glucose-stimulated IRE1α activation in pancreatic β-cells. *Sci. Signal.* **3,** ra7 (2010).
114. Lu, G. *et al.* PPM1l encodes an inositol requiring-protein 1 (IRE1) specific phosphatase that regulates the functional outcome of the ER stress response. *Mol. Metab.* **2,** 405–416 (2014).
115. Ren, S. *et al.* IRE1 phosphatase PP2Ce regulates adaptive ER stress response in the postpartum mammary gland. *PLoS ONE* **9,** e111606 (2014).
116. Gu, F. *et al.* Protein-tyrosine phosphatase 1B potentiates IRE1 signaling during endoplasmic reticulum stress. *J. Biol. Chem.* **279,** 49689–49693 (2004).
117. Jwa, M. & Chang, P. PARP16 is a tail-anchored endoplasmic reticulum protein required for the PERK- and IRE1α-mediated unfolded protein response. *Nat. Cell Biol.* **14,** 1223–1230 (2012).
118. Zhu, X. *et al.* Ubiquitination of Inositol-requiring Enzyme 1 (IRE1) by the E3 Ligase CHIP Mediates the IRE1/TRAF2/JNK Pathway. *J. Biol. Chem.* **289,** 30567–30577 (2014).
119. Gade, P. *et al.* Regulation of the death-associated protein kinase 1 expression and autophagy via ATF6 requires apoptosis signal-regulating kinase 1. *Mol. Cell Biol.* **34,** 4033–4048 (2014).
120. Lee, J. *et al.* p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. *Nat. Med.* **17,** 1251–1260 (2011).
121. Wang, F. M. & Ouyang, H. J. Regulation of unfolded protein response modulator XBP1s by acetylation and deacetylation. *Biochem. J.* **433,** 245–252 (2010).
122. Chen, H. & Qi, L. SUMO modification regulates the transcriptional activity of XBP1. *Biochem. J.* **429,** 95–102 (2010).
123. Ameri, K. & Harris, A. L. Activating transcription factor 4. *Int. J. Biochem. Cell Biol.* **40,** 14–21 (2008).
124. Chow, C. Y., Wang, X., Riccardi, D., Wolfner, M. F. & Clark, A. G. The genetic architecture of the genome-wide transcriptional response to ER stress in the mouse. *PLoS Genet.* **11,** e1004924 (2015).
125. Dombroski, B. A. *et al.* Gene expression and genetic variation in response to endoplasmic reticulum stress in human cells. *Am. J. Hum. Genet.* **86,** 719–729 (2010).
126. Sakaki, K. *et al.* RNA surveillance is required for endoplasmic reticulum homeostasis. *Proc. Natl Acad. Sci. USA* **109,** 8079–8084 (2012).
127. Carreras-Sureda, A. & Hetz, C. RNA metabolism: Putting the brake on the UPR. *EMBO Rep.* **16,** 545–546 (2015).
128. Karam, R. *et al.* The unfolded protein response is shaped by the NMD pathway. *EMBO Rep.* **16,** 599–609 (2015).
129. Raina, K. *et al.* Targeted protein destabilization reveals an estrogen-mediated ER stress response. *Nat. Chem. Biol.* **10,** 957–962 (2014).
130. Genereux, J. C. *et al.* Unfolded protein response-induced ERdj3 secretion links ER stress to extracellular proteostasis. *EMBO J.* **34,** 4–19 (2014).
131. Cooley, C. B. *et al.* Unfolded protein response activation reduces secretion and extracellular aggregation of amyloidogenic immunoglobulin light chain. *Proc. Natl Acad. Sci. USA* **111,** 13046–13051 (2014).
132. Moenner, M., Pluquet, O., Bouchecareilh, M. & Chevet, E. Integrated endoplasmic reticulum stress responses in cancer. *Cancer Res.* **67,** 10631–10634 (2007).
133. Ilani, T. *et al.* A secreted disulfide catalyst controls extracellular matrix composition and function. *Science* **341,** 74–76 (2014).
134. Dihazi, H. *et al.* Secretion of ERP57 is important for extracellular matrix accumulation and progression of renal fibrosis, and is an early sign of disease onset. *J. Cell Sci.* **126,** 3649–3663 (2014).
135. Martinon, F., Chen, X., Lee, A. H. & Glimcher, L. H. TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat. Immunol.* **11,** 411–418 (2010).
136. Woo, C. W. *et al.* Adaptive suppression of the ATF4-CHOP branch of the unfolded protein response by toll-like receptor signalling. *Nat. Cell Biol.* **11,** 1473–1480 (2009).
137. Woo, C. W., Kutzler, L., Kimball, S. R. & Tabas, I. Toll-like receptor activation suppresses ER stress factor CHOP and translation inhibition through activation of eIF2B. *Nat. Cell Biol.* **14,** 192–200 (2012).
138. Reimold, A. M. *et al.* Plasma cell differentiation requires the transcription factor XBP-1. *Nature* **412,** 300–307 (2001).
139. Hu, C. C., Dougan, S. K., McGehee, A. M., Love, J. C. & Ploegh, H. L. XBP-1 regulates signal transduction, transcription factors and bone marrow colonization in B cells. *EMBO J.* **28,** 1624–1636 (2009).
140. Huh, W. J. *et al.* XBP1 controls maturation of gastric zymogenic cells by induction of MIST1 and expansion of the rough endoplasmic reticulum. *Gastroenterology* **139,** 2038–2049 (2010).
141. Lipson, K. L. *et al.* Regulation of insulin biosynthesis in pancreatic beta cells by an endoplasmic reticulum-resident protein kinase IRE1. *Cell Metab.* **4,** 245–254 (2006).
142. Villeneuve, J. *et al.* A protective role for CD154 in hepatic steatosis in mice. *Hepatology* **52,** 1968–1979 (2010).
143. Karali, E. *et al.* VEGF Signals through ATF6 and PERK to promote endothelial cell survival and angiogenesis in the absence of ER stress. *Mol. Cell* **54,** 559–572 (2014).
144. Verfaillie, T. *et al.* PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. *Cell Death Differ.* **19,** 1880–1891 (2012).
145. Munoz, J. P. *et al.* Mfn2 modulates the UPR and mitochondrial function via repression of PERK. *EMBO J.* **32,** 2348–2361 (2013).
146. Ngoh, G. A., Papanicolaou, K. N. & Walsh, K. Loss of mitofusin 2 promotes endoplasmic reticulum stress. *J. Biol. Chem.* **287,** 20321–20332 (2012).
147. Mori, T., Hayashi, T., Hayashi, E. & Su, T. P. Sigma-1 receptor chaperone at the ER-mitochondrion interface mediates the mitochondrion-ER-nucleus signaling for cellular survival. *PLoS ONE* **8,** e76941 (2013).
148. Son, S. M., Byun, J., Roh, S. E., Kim, S. J. & Mook-Jung, I. Reduced IRE1α mediates apoptotic cell death by disrupting calcium homeostasis via the InsP3 receptor. *Cell Death Dis.* **5,** e1188 (2014).
149. Arruda, A. P. *et al.* Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat. Med.* **20,** 1427–1435 (2014).
150. Mardones, P., Martinez, G. & Hetz, C. Control of systemic proteostasis by the nervous system. *Trends Cell Biol.* **25,** 1–10 (2014).
151. Taylor, R. C. & Dillin, A. XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. *Cell* **153,** 1435–1447 (2013).
152. Williams, K. W. *et al.* Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis. *Cell Metab.* **20,** 471–482 (2014).
153. Hetz, C., Chevet, E. & Harding, H. P. Targeting the unfolded protein response in disease. *Nat. Rev. Drug Discov.* **12,** 703–719 (2013).

Erratum: Proteostasis control by the unfolded protein response

Claudio Hetz, Eric Chevet and Scott A. Oakes

Nat. Cell Biol. **17,** 829–838 (2015); published online 30 June 2015; corrected after print 13 July 2015

In the version of this Review originally published, Fig. 1 contained a superfluous IRE1α stress sensor at the top of the schematic. This error has been corrected in all online versions.