Trends in Pharmacological Sciences

CellPress REVIEWS

Review

Small Molecules to Improve ER Proteostasis in Disease

Vicente Gonzalez-Teuber,[1,2,3,8] Hector Albert-Gasco,[4,8] Vincent C. Auyeung,[5,6,8] Feroz R. Papa,[5,*] Giovanna R. Mallucci,[4,*] and Claudio Hetz[1,2,3,7,*]

Abnormally high levels of misfolded proteins in the endoplasmic reticulum (ER) lumen result in a stress state that contributes to the progression of several pathological conditions including diabetes, cancer, neurodegeneration, and immune dysregulation. ER stress triggers a dynamic signaling pathway known as the unfolded protein response (UPR). The UPR enforces adaptive or cell death programs by integrating information about the intensity and duration of the stress stimuli. Thus, depending on the disease context, ER stress signaling can be beneficial or detrimental. We discuss current efforts to develop small molecules to target distinct components of the UPR, and their possible applications in treating human disease, focusing on neurodegenerative diseases, metabolic disorders, and cancer.

Highlights

ER stress induces a cellular reaction, the UPR, to restore cellular proteostasis.

Chronic ER stress is a hallmark in the pathology of several human diseases including cancer, obesity, neurodegeneration, and diabetes.

Small molecules to target UPR mediators promise efficacy for the treatment of many human diseases.

ER Proteostasis Control

The function of a protein depends on its tertiary structure, a highly intricate 3D arrangement. Protein folding occurs for more than ten thousand different protein species in parallel [1], and one third of the proteome is folded and processed through the secretory pathway. This involves additional challenges because proteins often contain several hydrophobic transmembrane domains that tend to aggregate. The endoplasmic reticulum (ER) operates as a central site to execute folding and quality control of newly synthesized proteins. To assist protein folding, a plethora of foldases, oxidoreductases, and processing enzymes are expressed inside the ER to maintain **proteostasis** (see Glossary) [2]. In addition, the folding process requires a tightly controlled redox and ionic environment. The folding capacity of the ER can be compromised under physiological and pathological circumstances, resulting in a cellular state known as **ER stress**. To cope with protein folding stress, a signaling reaction has evolved to adjust the protein folding demand according to needs in a dynamic manner, a pathway termed the **unfolded protein response** (UPR) (reviewed in [3]).

The mammalian UPR is governed by three stress transducers located at the ER membrane that sense protein folding status. These signal transducers act in concert to integrate stress signals to enforce gene expression programs and cytosolic responses to restore proteostasis. In doing so, UPR activation increases the folding capacities of the ER, triggers ER and Golgi biogenesis and protein degradation, and attenuates protein synthesis, among other reactions. If cells fail to adapt, the UPR leads to an apoptotic response, thus determining cell fate under chronic ER stress (reviewed in [4,5]).

In the past decade ER stress and/or overactivation of UPR signaling have emerged as a prominent feature of several pathological conditions including diabetes mellitus, obesity, fibrosis, neurodegenerative diseases, ischemia, chronic inflammation, liver disease, and cancer (reviewed in [6]). The UPR has become an attractive target in the design of novel treatments, leading to the discovery of novel small molecules that can target spe-

[1]Biomedical Neuroscience Institute (BNI), Faculty of Medicine, University of Chile, Santiago, Chile
[2]Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP) Center for Geroscience, Brain Health, and Metabolism, Santiago, Chile
[3]Program of Cellular and Molecular Biology, Institute of Biomedical Science, University of Chile, Santiago, Chile
[4]UK Dementia Research Institute at the University of Cambridge and Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK
[5]Department of Medicine, Department of Pathology, Diabetes Center, Lung Biology Center, and University of California San Francisco (UCSF) Quantitative Bioscience Institute, San Francisco, CA, USA
[6]Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Medicine, University of California, San Francisco, CA, USA
[7]Buck Institute for Research on Aging, Novato, CA, USA
[8]Equal contributions

*Correspondence:
frpapa@medicine.ucsf.edu (F.R. Papa), gm522@cam.ac.uk (G.R. Mallucci), and chetz@med.uchile.cl (C. Hetz).

cific components of the pathway. In this article we discuss recent efforts to modulate the UPR using pharmacological approaches and their possible applications in disease treatment.

UPR Signaling

Three main stress transducers initiate the UPR, including activating transcription factor 6 (ATF6 α and β isoforms), protein kinase RNA-like ER kinase (PERK), and inositol-requiring enzyme (IRE1 α and β isoforms) (Figure 1) (reviewed in [4]). IRE1α is a type I transmembrane ER-resident protein, composed of an ER luminal sensor domain and, facing the cytoplasm, a serine/threonine kinase domain and an RNase domain. Two models have been proposed to control the initiation of the UPR (reviewed in [7]): an indirect model in which, under non-stress conditions, UPR sensors are repressed by binding to the ER **chaperone** BiP (also known as GRP78), which is released under ER stress. By contrast, a direct recognition model has been proposed for IRE1α and PERK in which they bind to unfolded proteins as ligands to induce their activation. IRE1 signaling involves its dimerization and *trans*-autophosphorylation, leading to a conformational change that activates its RNase domain (Figure 2) (reviewed in [3]). Structural studies have demonstrated that the IRE1α kinase and RNase domains assemble into a back-to-back dimer and higher-order oligomers (Figure 2). IRE1α processes the mRNA encoding the transcription factor X-box binding protein 1 (XBP1), excising a 26 nucleotide intron in the *XBP1* mRNA, followed by exon–exon ligation by the tRNA ligase RTCB [5]. This unconventional splicing event shifts the coding reading frame, leading to the translation of a potent transcription factor termed XBP1s [4]. XBP1s controls the transcription of genes involved in lipid synthesis, protein folding, **ER-associated degradation** (ERAD), and protein translocation into the ER, among other targets [5]. IRE1α can also diversify the targets of its RNase activity through a process known as regulated IRE1-dependent decay (RIDD) (reviewed in [8]). RIDD reduces the stability of ER-localized mRNAs, miRNAs, and rRNAs through cleavage of a specific sequence [9]. Although controversial, the ratio between RIDD and *XBP1* mRNA splicing may depend on the oligomerization state of IRE1α [5]. IRE1α can also act as a scaffold for signal transduction through the binding to adapter proteins and regulatory components, a platform referred to as the UPRosome [10]. IRE1α-binding partners mediate the crosstalk with other signaling pathways, including JUN N-terminal kinase (JNK) and apoptosis signal-regulating kinase 1 (ASK1), nuclear factor κ light chain enhancer of activated B cells (NF-κB) (reviewed in [11]), and the cytoskeleton [12]. The intricacy of IRE1α activation affords multiple opportunities to chemically modulate its various activities (reviewed in [13]) (Box 1).

PERK is also a type I transmembrane protein that contains a kinase domain in its cytosolic region. Under ER stress, PERK undergoes a conformational change allowing its dimerization and *trans*-autophosphorylation. Activated PERK phosphorylates the eukaryotic translation initiation factor 2α (eIF2α). Phosphorylated eIF2α (P-eIF2α) binds tightly to eIF2B, preventing the formation of the larger complex, thus inhibiting the initiation of translation, shutting down protein synthesis, and therefore reducing the load of proteins at the ER [14]. However, some mRNAs can overcome this suppression through non-canonical translation initiation by using an **internal ribosome entry site** (IRES) element or **upstream open reading frames** (uORFs). The best example of increased translation induced by eIF2α phosphorylation is activating transcription factor 4 (ATF4) is the best example of increased translation induced by eIF2α phosphorylation, which controls the expression of the proapoptotic CCAAT/enhancer-binding protein homologous protein (CHOP) (reviewed in [15]). Together, the two transcription factors control oxidative stress responses, peptide synthesis, autophagy, and apoptosis [2]. Once ER stress is relieved and protein homeostasis is restored, ATF4 and CHOP induce the expression of DNA damage-inducible protein 34 (GADD34), which dephosphorylates P-eIF2α

Glossary

Chaperone: proteins that assist protein folding, preventing their aggregation.
ER-associated degradation (ERAD): a pathway which targets and eliminate misfolded proteins of the endoplasmic reticulum (ER) though ubiquitination, retrotranslocation to the cytosol, and degradation by the proteasome.
ER stress: a condition where the folding capacities of the ER are compromised, leading to the accumulation of abnormal levels of misfolded proteins.
Integrated stress response (ISR): stress signaling pathways that converge on the phosphorylation of eukaryotic translation factor 2α (eIF2α), decreasing global protein synthesis.
Proteostasis: a cluster of biological pathways that control the biogenesis, folding, trafficking, and degradation of proteins at the cellular level.
Internal ribosome entry sites (IRESs) and upstream open reading frames (uORFs): mRNA elements that enable mRNA translation in a 5′-cap-independent manner.
Unfolded protein response (UPR): a set of signaling reactions that are triggered by ER stress to restore proteostasis or induce the apoptosis of damaged cells.

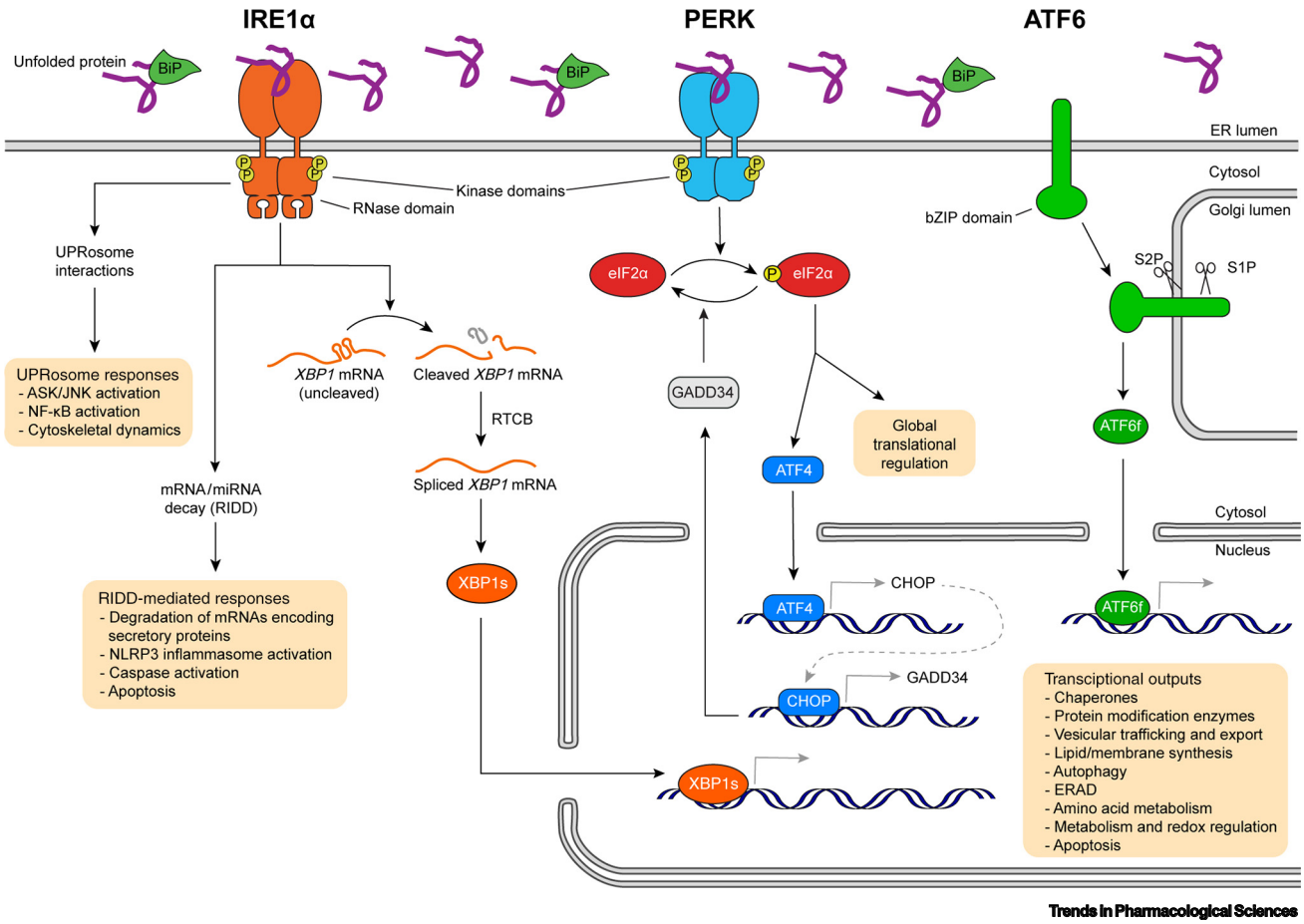

Figure 1. Unfolded Protein Response (UPR) Signaling Pathways. The burden of misfolded proteins in the lumen of the endoplasmic reticulum (ER) activates three UPR sensors: activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and protein kinase R-like ER resident kinase (PERK), triggering signaling pathways that aim to relieve ER stress conditions by increasing folding capacity and inhibiting protein synthesis, among others. Abbreviations: ASK, apoptosis signal-regulating kinase 1; bZIP, basic leucine zipper; CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein; eIF2α, eukaryotic initiation factor 2α; ERAD, ER-associated degradation; GADD34, DNA-damage-inducible protein 34; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor κB; NLRP3, NACHT, LRR and PYD domains-containing protein 3; P, phosphorylation; RIDD, IRE1-dependent decay; RTCB, tRNA ligase; S1P, site 1 protease; S2P, site 2 protease; XBP1, X-box binding protein 1.

to restore translation [15]. Importantly, PERK is part of a larger pathway termed the **integrated stress response** (ISR), where three additional kinases converge on the phosphorylation of eIF2α, including protein kinase R (PKR), heme-regulated eIF2α kinase (HRI), and general control nonderepressible 2 kinase (GCN2) (reviewed in [15]).

ATF6 is also a transmembrane ER-resident protein that translocates to the Golgi apparatus under ER stress where it is cleaved by transmembrane site 1 and site 2 proteases to release a cytosolic fragment containing a basic leucine zipper (bZIP) transcription factor (ATF6f) (reviewed in [16]). Activation of ATF6 involves the release of BiP binding from an ER-to-Golgi translocation signal. ATF6 is also regulated by its glycosylation and redox state, in addition to the binding of protein disulfide isomerases (PDI) (reviewed in [5]). ATF6f regulates the transcription of ER-resident chaperones, *XBP1*, and components of the ERAD machinery, among other targets.

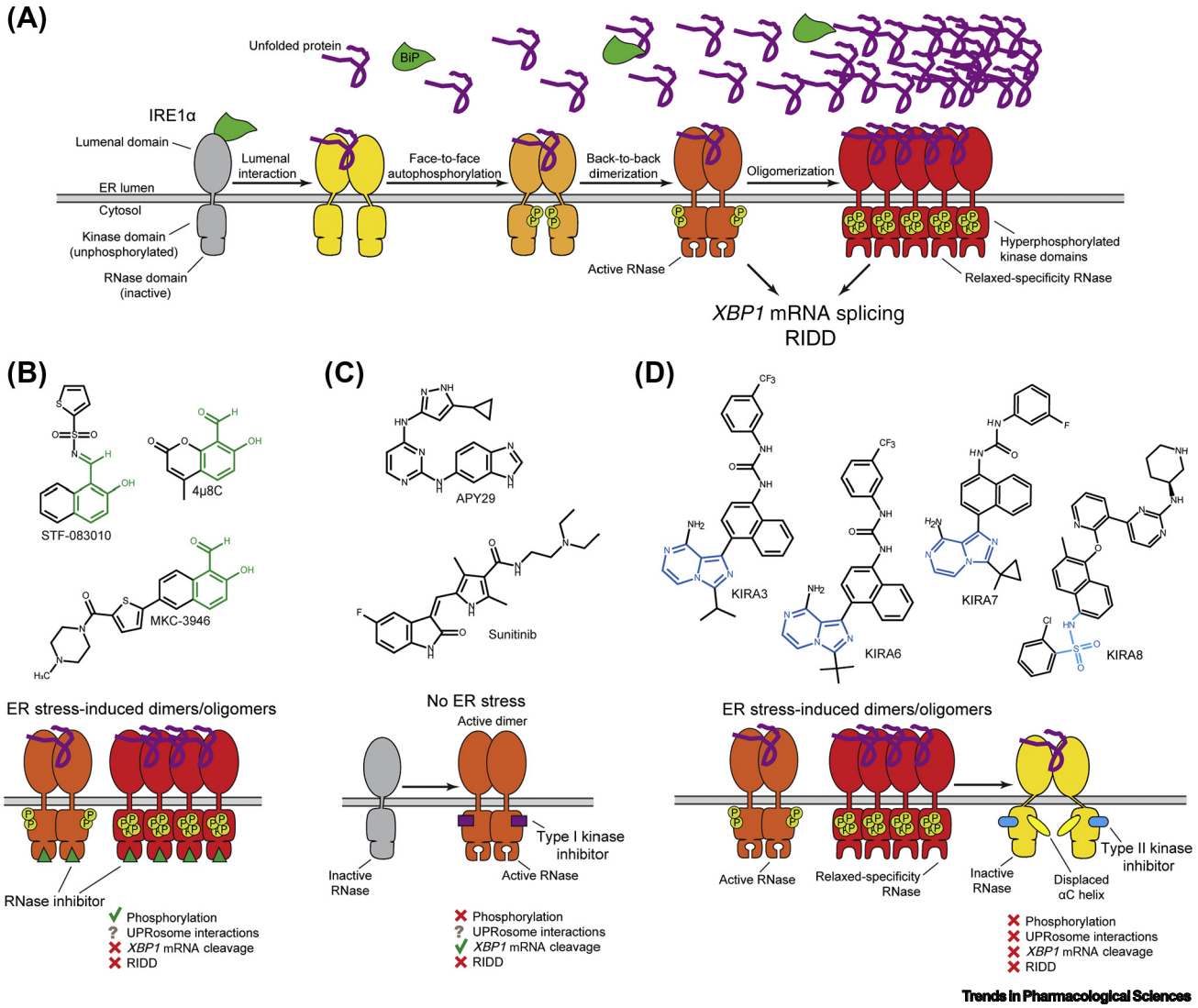

Figure 2. Chemical Modulators of IRE1α Activity. (A) Model for IRE1α activation. Unfolded proteins in the endoplasmic reticulum (ER) induce association of the luminal domains and the release of BiP to bind misfolded proteins, followed by dimerization and *trans*-autophosphorylation of the kinase domains. (B) RNase inhibitors of IRE1α contain a common salicylaldehyde pharmacophore (green) that forms a Schiff base with the IRE1α RNase active site. Kinase activity and dimerization/oligomerization are preserved. (C) Type I kinase inhibitors of IRE1α act as partial agonists and induce dimerization and RNase activity even in the absence of ER stress. (D) Type II kinase inhibitors stabilize an inactive conformation of the kinase, with displacement of the αC helix, thereby preventing back-to-back dimerization or oligomerization. Type II kinase inhibitors can block all known biological activities of IRE1α. Abbreviations: IRE1α, inositol-requiring enzyme 1α; P, phosphorylation; RIDD, IRE1-dependent decay; XBP1, X-box binding protein.

In summary, the three UPR signaling branches act in conjunction to coordinate a feedback response to mitigate protein misfolding (Figure 1), where this signaling pathway can either promote cell survival or engage a terminal phase by engaging proapoptotic pathways.

Targeting the UPR for Disease Intervention

Strategies to manipulate the UPR have been exploited to define possible links between ER stress and human disease, with great advances in cancer and neurodegeneration. The available data suggest a complex scenario wherein distinct signaling components of the UPR might have selective, and sometimes even opposing, consequences for disease progression. In the next sections

Box 1. Small Molecules to Inhibit IRE1α

Several inhibitors of the IRE1α RNase domain have been developed, exemplified by STF-083010, MKC-3946, and 8-formyl-7-hydroxy-4-methylcoumarin (4μ8C) (see Figure 2B in main text). The efficacy of these compounds is driven by reactivity rather than by binding affinity, especially because the IRE1α RNase pocket is both shallow and relatively polar. Kinase inhibitors of IRE1α can allosterically modulate the RNase catalytic activity and are more amenable for optimization to drug-likeness. Kinase inhibitors generally fall into (at least) two classes [77], but in IRE1α these distinct classes of kinase inhibitors have divergent effects on RNase activity. Type I inhibitors competitively engage the ATP binding pocket, and the resulting kinase–drug complex adopts a conformation that is similar to the active, ATP-bound conformation. Similarly, the nonspecific type I inhibitor APY29 and the broad-spectrum receptor tyrosine kinase inhibitor sunitinib induced *XBP1* mRNA cleavage activity by quantitatively dephosphorylated IRE1α, and induced dimerization (see Figure 2C in main text) [78]. Thus, type I kinase inhibitors actually behave as partial agonists of IRE1α RNase activity. Type II kinase inhibitors occupy a hydrophobic site adjacent to the ATP binding pocket and stabilize a distinct conformation. The earliest type II inhibitors were based on a pyrazolopyrimidine scaffold and were named kinase-inhibiting RNase attenuators (KIRAs) (see Figure 2D in main text) [26,79]. Since then, additional type II inhibitors have been developed on an imidazopyrazine scaffold, as well as a sulfonamide compound variously called compound 18 or KIRA8 – that is highly selective for IRE1α with nanomolar potency [17,80]. As expected of type II inhibitors, crystal structures show that KIRA compounds stabilize a conformation in the kinase domain activation loop. More importantly, these compounds also displace the kinase αC helix that disrupts the back-to-back dimer interface and thus prevents an RNase-active conformation [80]. This explains why KIRA compounds induce monomerization of IRE1α, and why KIRA-bound IRE1α is crippled for both *XBP1* mRNA splicing (catalyzed by the dimers and oligomers) and RIDD (catalyzed by oligomers). Thus, type II kinase inhibitors hold the potential for true rheostat control of IRE1α activity and permit chemical control over each of the individual activities catalyzed by IRE1α.

we summarize the design and use of small molecules to intervene in the UPR in conditions such as cancer, metabolic conditions, and neurodegenerative diseases.

Targeting IRE1α for Disease Intervention

Metabolic and Fibrotic Diseases

The protein folding capacity of professional secretory cells is overwhelmed in several diseases, leading to cell degeneration and death through terminal UPR signaling. For example, dysregulated UPR signaling in insulin-secreting pancreatic β cells results in premature cell loss, insulin deficiency, and diabetes. Type II inhibitors of IRE1α (Box 1) can delay progression or reverse diabetes in mouse models [17], illustrating the importance of IRE1α in the pathophysiology of this disease. In human idiopathic pulmonary fibrosis, dysregulated UPR signaling is thought to induce apoptosis in alveolar epithelial cells, the professional secretory cells that synthesize surfactant proteins in the lung. Administration of IRE1α inhibitors can prevent and reverse established fibrosis in a mouse model of pulmonary fibrosis [18].

Cancer

Tumor growth relies in the UPR as a selective force to drive malignant transformation [19], in addition to remodeling the tumor microenvironment and anticancer immune responses [20], as well as impacting on other central hallmarks of cancer [21]. Malignant cells derived from secretory cells, such as B cells in multiple myeloma and pancreatic adenocarcinoma cells, depend on XBP1s to cope with their intense secretory burden, and treatment with the IRE1α RNase inhibitors MKC-3946 or STF-083010 reduces tumor growth [22,23]. In 'triple-negative' breast cancer lacking estrogen, progesterone, and epidermal growth factor receptors, XBP1s promotes cancer cell growth, angiogenesis, and cell survival [24]. In mouse models of breast cancer, inhibition of the IRE1α RNase using STF-083010 or a newer-generation inhibitor MKC-8866 potentiated the efficacy of paclitaxel and tamoxifen *in vivo* [25,26]. In ovarian cancer, XBP1 function was implicated not in the malignant cells but instead in tumor-associated T cells. In this study, the tumor microenvironment in the form of malignant ascites fluid induced IRE1α and XBP1, which decreased mitochondrial respiration to impair effector T cell function. The IRE1α RNase inhibitor 4μ8C in T cells rescued these defects, and *Xbp1* deficiency increased tumor T cell infiltration and correspondingly decreased tumor growth and peritoneal metastasis [27]. In glioblastoma multiforme (GBM), a recent study suggested that *XBP1* mRNA splicing

and RIDD have divergent roles in the progression of glioblastoma, where expression of *XBP1* correlates with more aggressive cancer [28]. Interestingly, one somatic mutant form of IRE1α (P338L) appeared to have enhanced capacity to degrade miR-17, but, unlike the other somatic mutants studied, actually protected against tumor invasion [28]. Dominant-negative IRE1α was protective in mice, validating the concept of overall IRE1α inhibition, but these findings nonetheless highlight the importance of dissecting the nuances of IRE1α function in clinically relevant models of disease.

Retinal Disease

Although neurons are not typically considered to be classical secretory cells, the accumulation of abnormal aggregates may nonetheless induce the terminal UPR in neurons and lead to neurodegeneration (reviewed in [29]). ER stress has been implicated in ocular diseases and in the death of neuronal photoreceptor cells (reviewed in [30]). IRE1α inhibition by type II inhibitors protected against retinal cell death in a murine model of retinal degeneration [31]. In the context of neurodegenerative diseases, although genetic evidence has demonstrated a functional role of the IRE1/XBP1 pathway in multiple brain diseases (reviewed in [29,32]), so far no studies are available demonstrating the benefits of targeting this UPR branch with small molecules.

Modulation of the PERK–eIF2α Pathway for Disease Intervention

Several studies have highlighted the potential of inhibiting the PERK pathway and the ISR to confer neuroprotection and restore normal cognitive function through the restoration of neuronal protein synthesis rates (reviewed in [33,34]). Numerous small molecules have since been developed that can modulate this pathway with the aim of developing new therapeutics (Figure 3).

The first PERK inhibitors generated are GSK2606414 and GSK2656157, are highly potent kinase inhibitors with favorable pharmacokinetic properties, and were initially developed for the treatment of cancer [35,36]. These compounds bind to the kinase domain in the ATP binding site cleft [36] and strongly prevent eIF2α phosphorylation in the presence of ER stressors. In addition, strategies to sustain eIF2α phosphorylation have also uncovered interesting compounds. Salubrinal was identified as a general inhibitor of eIF2α phosphatases by screening compounds that can protect cells from ER stress-mediated cell death. Salubrinal is a nonselective inhibitor of both the constitutive CreP/PP1 and stress-induced GADD34/PP1 phosphatase complexes. Other studies attempted to identify selective inhibitors of the GADD34/PP1 phosphatase that would only target cells suffering from ER stress. This approach identified guanabenz and its derivative sephin-1 [37,38], both of which prolong the phosphorylation of eIF2α in the context of ER stress. Finally, the consequences of eIF2α phosphorylation can also be modulated by using compounds that act downstream of PERK and eIF2α. ISRIB is a small molecule that binds strongly to eIF2B [39,40] and partially restores protein synthesis by allowing ternary complex formation even in the presence of high levels of P-eIF2α (Box 2). Similarly, trazodone and dibenzoylmethane, two repurposed drugs uncovered in a screen of UPR inhibitors, also restore protein translation without changing eIF2α phosphorylation, although their precise mechanism of action is not yet clear [41].

Neurodegenerative Diseases

Markers of UPR activation have been extensively reported in the brain of patients affected with neurodegenerative diseases, in association with the accumulation of misfolded disease-specific proteins (reviewed in [29]). Phosphorylated PERK (P-PERK) and P-eIF2α have been reported in the brains of patients with Alzheimer disease (AD), Parkinson disease (PD), and amyotrophic lateral sclerosis (ALS) [42,43].

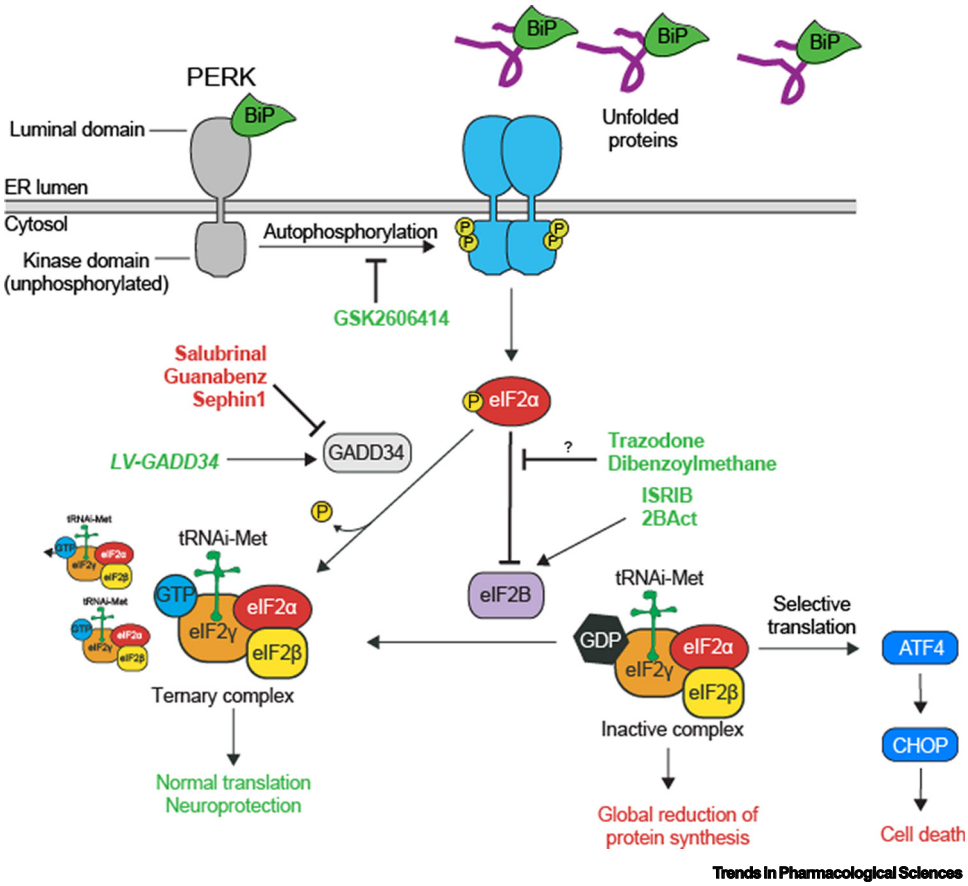

Figure 3. Small Molecules and Lentiviruses Targeting the PERK Arm of the Unfolded Protein Response. Unfolded proteins induce autophosphorylation of PERK, which in turn phosphorylates eIF2α. Phosphorylation of eIF2α (generating P-eIF2α) rapidly attenuates protein synthesis by reducing ternary complex formation. P-eIF2α also leads to an increase in the expression of a subset of mRNAs, including ATF4 and CHOP. The PERK inhibitor GSK2606414 and the compounds ISRIB, trazodone, and DBM inhibit PERK signaling at various points in the pathway, preventing neurodegeneration and improving memory in several experimental models. Salubrinal inhibits P-eIF2α dephosphorylation, exacerbating neurodegeneration in some but not all models. The purported GADD34 inhibitors sephin 1 and guanabenz also lead to neuroprotection in some models, although doubts over their specificity have been raised. Lentivirus-mediated overexpression of GADD34 can also improve memory and prevent neurodegeneration by inhibiting PERK signaling via dephosphorylation of P-eIF2α. Abbreviations: ATF4, activating transcription factor 4; CHOP, CCAAT/enhancer-binding protein homologous protein; DBM, dibenzoylmethane; eIF2α, eukaryotic initiation factor 2α; GADD34, DNA-damage-inducible protein 34; P, phosphorylation; PERK, protein kinase R-like ER-resident kinase.

Because the signaling outputs downstream of eIF2α/ATF4 regulate multiple cellular responses (i.e., folding, redox, protein translation, and apoptosis), the possible consequences of targeting the pathway for a specific disease context are difficult to predict. The contribution of PERK to several neurodegenerative diseases has been well characterized using genetic and pharmacological approaches. Daily oral treatment of prion-disease mice with GSK2606414 effectively reduced levels of P-PERK and P-eIF2α, and restored protein synthesis rates in the brain, despite ongoing prion replication and accumulation of misfolded prion protein [44]. Boosting global protein synthesis rates in the brain of models of neurodegeneration increased the production of synaptic proteins, thereby preventing synapse degeneration and augmenting motor and cognitive functions [45]. Critically, treatment with GSK2606414 resulted in marked neuroprotection throughout the brain, preventing the development of clinical signs and neuronal loss. In a model of frontotemporal dementia (FTD), GSK2606414 significantly lowered levels of PERK

Box 2. Translation Initiation Inhibition by ISRIB

mRNA translation is a complex process that comprises three steps – initiation, elongation, and termination. The PERK pathway inhibits the initiation phase by phosphorylating eIF2α. P-eIF2α inhibits eIF2B complex activity, reducing the amount of ternary complex and shutting down protein translation (Figure I). The small molecules ISRIB and 2BAct stabilize the activated state of eIF2B by holding the decameric complex together, allowing guanine nucleotide exchange despite eIF2α phosphorylation.

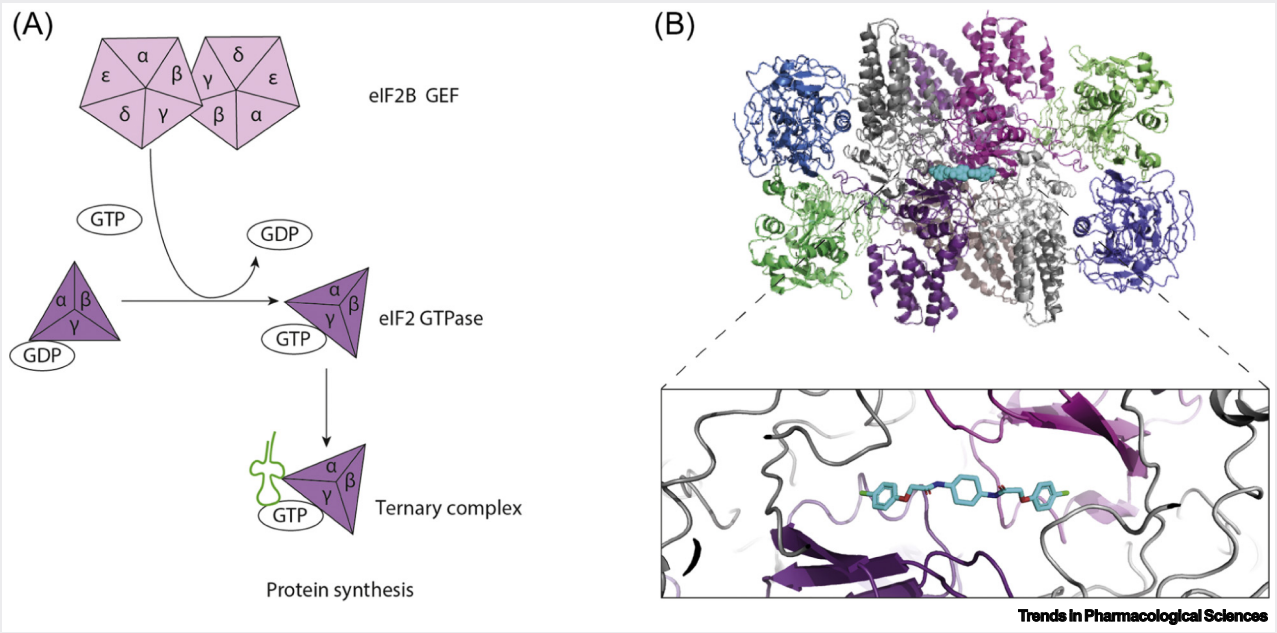

Figure I. Translation Initiation Inhibition by ISRIB. (A) Regulation of translational repression by eIF2B. eIF2B unloads GDP from eIF2, allowing it to form the ternary complex and restoring protein synthesis. (B) Cryo-electron microscopy structure of ISRIB (cyan space fill) bound to eIF2B at the interface of two pentamers composed of the α (purple), β (magenta), γ (blue), δ (grey), and ε (green) subunits that form the decamer complex. (C) Magnified view showing ISRIB (cyan) binding in the lipophilic pocket formed by the β and δ subunits. Structures are rendered using PyMOL (version 2.2.3) and the PDB coordinates 6EZO. Abbreviation: GEF, guanine exchange factor.

signaling, thereby restoring protein synthesis and avoiding further neuronal loss and progression of clinical disease [46]. Similar results were reported in a model of PD, where oral administration of GSK2606414 improved dopaminergic neuron survival associated with increased levels of dopamine in the brain, augmented expression of synaptic proteins, and improved motor performance [47]. However, direct PERK inhibition caused on-target toxicity to the pancreas, leading to acinar pancreatic cell death and weight loss [48]. This toxicity of PERK inhibition represents a major barrier to its therapeutic use. In addition, the specificity of GSK2606414 has been questioned because it can also inhibit the receptor-interacting serine/threonine-protein kinase (RIPK) [49], a central regulator of inflammatory processes and necroptosis.

Consistent with the pathogenic role of sustained phosphorylation of eIF2α in prion diseases, salubrinal was shown to accelerate disease progression in prion-infected mice, likely due to further lowering of global translation rates in brain and further repression of synaptic proteins [45]. These studies afford proof of principle that PERK pathway inhibition can prevent neurodegeneration, but new approaches were necessary to restore protein synthesis safely while maintaining effectiveness. The discovery of ISRIB indicated that restoring protein synthesis downstream of P-eIF2α improves memory formation in wild-type animals [50]. In prion-infected mice, ISRIB was neuroprotective, avoiding further neuronal loss without apparent pancreatic toxicity. This was due to a partial restoration of protein synthesis that was sufficient to avoid neuronal loss and restore associated cognitive processes without inducing pancreatic toxicity [48]. ISRIB

was recently shown to provide outstanding neuroprotection in a model of traumatic brain injury [51] as well as in a murine model of hearing loss [52]; however, it did not affect disease progression in an AD model [53,54]. An improved generation of ISRIB was recently generated, termed 2BAct, which was highly effective in treating models of vanishing white matter diseases, a neurodegenerative disease triggered by mutations in eIF2B, the direct ISRIB target [55]. Recent studies suggested that there is a defined window for the efficacy of ISRIB, where it may engage the target only at low levels of ISR activation [56]. ISRIB and 2BAct are highly specific and well-studied in terms of their molecular mechanisms of action. However, future efforts should focus on improving their solubility and defining the side effects of chronic administration.

The idea of sustaining eIF2α phosphorylation to treat particular diseases has also been exploited by targeting the regulatory subunits of the GADD34 phosphatase [38,57]. The rationale is that boosting UPR signaling to reduce misfolded protein overload may help to mitigate ER stress. Neuroprotective effects were reported in models of a rare genetic variant of ALS and in Charcot–Marie–Tooth type 1B (CMTB), a rare inherited peripheral neuropathy [58,59]. By contrast, another study indicated that guanabenz accelerates experimental ALS [60]. However, the selectivity and mechanism of action of the compounds used are debated [57,61]. Salubrinal also improved motor performance and reduced neurodegeneration in PD models based on α-synuclein overexpression [62]. It is likely that the opposing effects of the two approaches might result from several factors, including the timing of administration of the compound during the course of the disease, the organellar location of misfolded protein, and the levels of ER stress. CMTB mutant-related myelin proteins accumulate within the ER, whereas in most neurodegenerative diseases the misfolded proteins accumulate in the cytosol, interfering with global secretory proteostasis via various sites of action (reviewed in [29]).

A drug screen of a NINDS library of 1040 compounds (75% of which are licensed drugs) in a *C. elegans* model of UPR overactivation uncovered two compounds with similar biological effects to ISRIB [41], which inhibited P-eIF2α downstream signaling while leaving P-eIF2α levels unaffected. The compounds, the approved antidepressant trazodone and dibenzoylmethane (DBM) were then used in a FTD model and in prion-infected mice, and in both cases promoted a partial restoration of protein synthesis by restoring the levels of the ternary complex [41]. Trazodone and DBM treatment extended the lifespan of and provided remarkable neuroprotection in prion-infected mice and FTD animals, with significant prevention of neuronal loss [41]. Collectively, these data highlight the importance of the PERK–eIF2α pathway in neurodegeneration.

Cancer and Metabolic Disorders

PERK inhibitors have been tested as anticancer agents and displayed high efficacy in human xenograft models [27]. In addition, *in vitro* studies using salubrinal, guanabenz, or ISRIB have indicated ab impact on malignant disease phenotypes such as invasion and metastasis [63,64]. ISRIB was recently reported to have strong therapeutic effects in a model of prostate cancer [65]. This protective effect is thought to take place through P-eIF2α acting as a translational efficiency modulator, dampening the immune response. Further experiments using small molecules will give more insights into potential applications in clinical oncology.

PERK is also pathologically overactivated in a series of metabolic disorders such as diabetes, non-alcoholic fatty acid disease, and cardiovascular conditions. In line with these findings, chronic ER stress causes insulin resistance and contributes to liver dysfunction (reviewed in [66]). However, studies addressing the consequences of targeting PERK signaling with small molecules in metabolic disease are still lacking. A recent report showed that low doses of the

PERK inhibitor GSK2656157 increased glucose-stimulated insulin secretion, whereas higher levels of the inhibitor abolished insulin release because of pancreatic toxicity [67].

ATF6 Modulators

Screens using cell culture models of ER stress found compounds that modulate ATF6α. Ceapins are potent inhibitors of ATF6α activity [68], and are proposed to ablate the transport of ATF6α from the ER to the Golgi apparatus [69]. Activators of ATF6 signaling were also identified, known as compounds 147 and 263 [70,71]. Compound 147 was recently shown to covalently modify protein disulfide isomerases (PDIs) to promote ATF6α activation [71]. Remarkably, treatment with compound 147 achieved favorable plasma concentrations and protected tissue damage in various models of ischemia, supporting its therapeutic potential [72]. Although targeting ATF6 has great potential for disease intervention, more preclinical studies will be necessary to define further applications of ATF6 modulation.

Conclusions and Future Perspectives

UPR signaling plays a central role in determining the fate of cells suffering from ER stress by allowing the repair of the damage, thereby sustaining proteostasis through multiple cellular outputs, or inducing cell destruction programs through apoptosis. These two extremes of UPR activation are of intrinsic relevance to the progression of various diseases, in addition to being fundamental in maintaining normal cell function in many organs, as well as in embryonic development (reviewed in [73]). However, it is predicted that, depending on the disease context, modifying UPR signaling with small molecules may provide therapeutic effects by modulating specific cellular outputs (i.e., secretion, protein synthesis, ERAD, autophagy, apoptosis). Reducing the adaptive signaling of the UPR may be useful in treating cancer and reducing the growth of solid tumors and hematologic malignancies, as well as improving immune responses, whereas increasing the repair capacity of the UPR could translate into benefits in a variety of metabolic and neurodegenerative diseases. In the brain, abnormal levels of P-eIF2α are likely to be detrimental in many neurodegenerative conditions because of impaired synthesis of synaptic and other key proteins affecting neuronal function and survival. The kinetic behavior of the individual UPR transducers differs between them, and prolonged or unresolved ER stress turns off *XBP1* mRNA splicing to inactivate the prosurvival consequences of XBP1s expression. This, in combination with sustained PERK activation, translates into a strong degenerative program mediated by the production of reactive oxygen species, sustained inhibition of protein translation, and engagement of apoptosis [4].

Additional applications of UPR-targeting drugs need to be addressed in processes that may not be directly related to proteostasis impairment. Because the UPR is emerging as a key mediator of immune system regulation [74], the possible efficacy of ER stress-targeting compounds in autoimmune diseases needs to be further explored. The same is valid for conditions that alter cell differentiation, metabolism, and energy control [73]. Further, non-canonical PERK or IRE1α activation may occur in neurodegeneration by cytoplasmic (not ER) misfolded protein accumulation, as it is also seen in inflammation, angiogenesis, and metabolic control (reviewed in [75]). Similarly, neurotrophic signaling, like brain-derived neurotrophic factor (BDNF), can engage IRE1α and XBP1 to improve synaptic plasticity independently of ER stress [76]. The concept of the UPRosome suggests that many factors can control the amplitude and signaling kinetics of UPR sensors as well as their possible activation in the absence of ER stress [10]. A key concern is that small molecules that modulate the UPR and the ISR risk on-target side effects by modulating the UPR that is necessary for normal physiology. However, comparison between ISRIB and PERK inhibitors indicates that partial inhibition is safe and provides effective neuroprotection. Extensive

Outstanding Questions

Why is ER stress a common or universal feature of most brain diseases?

What are the possible side effects of UPR targeting drugs in non-human primates?

What is the efficacy of the combinatorial therapy of UPR inhibitors and classical chemotherapeutic agents in cancer clinical trials?

Because protein translation control is essential for the function of the brain, what are the consequences for our normal cognitive functions of chronic administration of small molecules that target the ISR?

Are these drugs likely to alter our memories, our personality, or any aspect that defines us as a person?

Is it possible to identify a biomarker to monitor ER stress levels in clinical trials to aid future testing of small molecules?

What are the consequences of combining low concentrations of PERK and IRE1α inhibitors in treating cancer?

studies with UPR-targeting drugs will be necessary to assess possible side effects upon long-term administration (see Outstanding Questions).

In conclusion, there is great potential for treating multiple human diseases through UPR modulation. Many small molecules that modify ER stress signaling are well tolerated in mice over months, with favorable pharmacokinetics. In the short term it is expected that the most promising UPR-targeting drugs will move into clinical trials in cancer, where combinatorial treatment with current chemotherapies may result in strong synergism and potency. In addition, targeting the UPR for the treatment of neurodegenerative diseases such as AD and PD, particularly with repurposed drugs, is imminent. Overall, the field has witnessed an exponential development in the discovery and optimization of small molecules targeting the UPR, setting the foundation for the development of therapeutics for the most common diseases affecting the human population.

Acknowledgments

Because of space limitations, we apologize that most primary papers in the introductory parts could not be cited and have been replaced by reviews. This work was funded by FONDECYT (Fondo Nacional de Desarrollo Científico y Tecnológico; grant 1140549), FONDAP (15150012), Millennium Institute (P09-015-F), the European Commission (MSCA-RISE 734749), Michael J. Fox Foundation for Parkinson's Research (9277), Fondo de Fomento al Desarrollo Científico y Tecnológico (FONDEF; ID16I10223 and D11E1007), US Office of Naval Research-Global (ONG-G; N62909-16-1-2003), US Air Force Office of Scientific Research (FA9550-16-1-0384), and the Muscular Dystrophy Association (to C.H.); European Research Council (ERC) consolidator award (UPR NEURO 647479), UK Dementia Research Institute, funded by UK DRI Ltd, Award UKDRI-2001; funders UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK, Wellcome Trust (collaborative award 201487/Z/16/Z), and Joint Programme Neurodegenerative Disease (JPND; MR/R024820/1) (to G.R.M); and the National Heart Lung and Blood Institute (1F32HL145990-01) (to V.C.A.).

References

1. Hartl, F. *et al.* (2011) Molecular chaperones in protein folding and proteostasis. *Nature* 475, 324–332
2. Balch, W.E. *et al.* (2008) Adapting proteostasis for disease intervention. *Science* 319, 916–919
3. Wang, M. and Kaufman, R.J. (2016) Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature* 529, 326–335
4. Walter, P. and Ron, D. (2011) The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334, 1081–1086
5. Hetz, C. and Papa, F.R. (2018) The unfolded protein response and cell fate control. *Mol. Cell* 69, 169–181
6. Almanza, A. *et al.* (2019) Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications. *FEBS J.* 2, 241–278
7. Karagöz, G.E. *et al.* (2019) The unfolded protein response: detecting and responding to fluctuations in the protein-folding capacity of the endoplasmic reticulum. *Cold Spring Harb. Perspect. Biol.* Published online January 22, 2019. https://doi.org/10.1101/cshperspect.a033886
8. Maurel, M. *et al.* (2014) Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* 39, 245–254
9. Oikawa, et al. (2010) Identification of a consensus element recognized and cleaved by IRE1. *Nucleic Acids Res.* 38, 6265–6273
10. Hetz, C. and Glimcher, L.H. (2009) Fine-tuning of the unfolded protein response: assembling the IRE1α interactome. *Mol. Cell* 35, 551–561
11. Hetz, C. (2012) The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.* 13, 89–102
12. Urra, H. *et al.* (2018) IRE1α governs cytoskeleton remodelling and cell migration through a direct interaction with filamin A. *Nat. Cell Biol.* 20, 942–953
13. Maly, D.J. and Papa, F.R. (2014) Druggable sensors of the unfolded protein response. *Nat. Chem. Biol.* 11, 892–901
14. Harding, H.P. *et al.* (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* 6, 1099–1108
15. Pakos-Zebrucka, K. *et al.* (2016) The integrated stress response. *EMBO Rep.* 17, 1374–1395
16. Hillary, R.F. and FitzGerald, U. (2018) A lifetime of stress: ATF6 in development and homeostasis. *J. Biomed. Sci.* 25, 48
17. Morita, S. *et al.* (2017) Targeting ABL–IRE1α signaling spares ER-stressed pancreatic β cells to reverse autoimmune diabetes. *Cell Metab.* 25, 883–897
18. Thamsen, M. *et al.* (2019) Small molecule inhibition of IRE1α kinase/RNase has anti-fibrotic effects in the lung. *PLoS One* 14, e0209824
19. Cubillos-Ruiz, J.R. *et al.* (2016) Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. *Cell* 168, 692–706
20. Rubio-Patiño, C. *et al.* (2018) Reshaping the immune tumor microenvironment through IRE1 signaling. *Trends Mol. Med.* 24, 607–614
21. Urra, H. *et al.* (2016) Endoplasmic reticulum stress and hallmarks of cancer. *Trends Cancer* 2, 252–262
22. Papandreou, I. *et al.* (2011) Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood* 117, 1311–1314
23. Mimura, N. *et al.* (2012) Blockade of XBP1 splicing by inhibition of IRE1 is a promising therapeutic option in multiple myeloma. *Blood* 119, 5772–5781
24. Chen, X. *et al.* (2014) XBP1 promotes triple-negative breast cancer by controlling the HIF1α pathway. *Nature* 508, 103–107
25. Ming, J. *et al.* (2015) A novel chemical, STF-083010, reverses tamoxifen-related drug resistance in breast cancer by inhibiting IRE1/XBP1. *Oncotarget* 6, 40692–40703
26. Logue, S.E. *et al.* (2018) Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. *Nat. Commun.* 9, 3267
27. Song, M. *et al.* (2018) IRE1α–XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. *Nature* 562, 423–428
28. Lhomond, S. *et al.* (2018) Dual IRE1 RNase functions dictate glioblastoma development. *EMBO Mol. Med.* 10, e7929

29. Hetz, C. and Saxena, S. (2017) ER stress and the unfolded protein response in neurodegeneration. *Nat. Rev. Neurol.* 13, 477–491
30. Kroeger, H. *et al.* (2019) ER stress and unfolded protein response in ocular health and disease. *FEBS J.* 286, 399–412
31. Ghosh, R. *et al.* (2014) Allosteric inhibition of the IRE1 a RNase preserves cell viability and function during endoplasmic reticulum stress. *Cell* 158, 534–554
32. Scheper, W. and Hoozemans, J.J.M. (2015) The unfolded protein response in neurodegenerative diseases: a neuropathological perspective. *Acta Neuropathol.* 130, 315–331
33. Buffington, S.A. *et al.* (2014) Translational control in synaptic plasticity and cognitive dysfunction. *Annu. Rev. Neurosci.* 37, 17–38
34. Martinez, G. *et al.* (2018) ER proteostasis control of neuronal physiology and synaptic function. *Trends Neurosci.* 9, 610–624
35. Axten, M. *et al.* (2012) Discovery of 7-methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3- dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). *J. Med. Chem.* 55, 7193–7207
36. Axten, M. *et al.* (2013) Discovery of GSK2656157: an optimized PERK inhibitor selected for preclinical development. *J. Med. Chem.* 4, 964–968
37. Das, I. *et al.* (2015) Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science* 348, 239–242
38. Tsaytler, P. *et al.* (2011) Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science* 332, 91–94
39. Tsai, J.C. *et al.* (2018) Structure of the nucleotide exchange factor eIF2B reveals mechanism of memory-enhancing molecule. *Science* 359, eaaq0939
40. Zyryanova, A.F. *et al.* (2018) Binding ISRIB reveals a regulatory site in the nucleotide exchange factor eIF2B. *Science* 359, 1533–1536
41. Halliday, M. *et al.* (2017) Repurposed drugs targeting eIF2α-P-mediated translational repression prevent neurodegeneration in mice. *Brain* 140, 1768–1783
42. Hoozemans, J.J.M. *et al.* (2007) Activation of the unfolded protein response in Parkinson's disease. *Biochem. Biophys. Res. Commun.* 354, 707–711
43. Smith, H.L. and Mallucci, G.R. (2016) The unfolded protein response: mechanisms and therapy of neurodegeneration. *Brain* 139, 2113–2121
44. Moreno, J.A. *et al.* (2013) Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Sci. Transl. Med.* 5, 206ra138
45. Moreno, J.A. *et al.* (2012) Sustained translational repression by eIF2α-P mediates prion neurodegeneration. *Nature* 485, 507–511
46. Radford, H. *et al.* (2015) PERK inhibition prevents tau-mediated neurodegeneration in a mouse model of frontotemporal dementia. *Acta Neuropathol.* 130, 633–642
47. Mercado, G. *et al.* (2018) Targeting PERK signaling with the small molecule GSK2606414 prevents neurodegeneration in a model of Parkinson's disease. *Neurobiol. Dis.* 112, 136–148
48. Halliday, M. *et al.* (2015) Partial restoration of protein synthesis rates by the small molecule ISRIB prevents neurodegeneration without pancreatic toxicity. *Cell Death Dis.* 6, e1672
49. Rojas-Rivera, D. *et al.* (2017) When PERK inhibitors turn out to be new potent RIPK1 inhibitors: critical issues on the specificity and use of GSK2606414 and GSK2656157. *Cell Death Differ.* 24, 1100–1110
50. Sidrauski, C. *et al.* (2013) Pharmacological brake-release of mRNA translation enhances cognitive memory. *eLife* 2, e00498
51. Chou, A. *et al.* (2017) Inhibition of the integrated stress response reverses cognitive deficits after traumatic brain injury. *Proc. Natl. Acad. Sci.* 114, E6420–E6426
52. Li, J. *et al.* (2018) Deletion of Tmtc4 activates the unfolded protein response and causes postnatal hearing loss. *J. Clin. Invest.* 128, 5150–5162
53. Johnson, E.C. *et al.* (2016) A small molecule targeting protein translation does not rescue spatial learning and memory deficits in the hAPP-J20 mouse model of Alzheimer's disease. *Peer J.* 4, e2565
54. Briggs, D.I. *et al.* (2017) Role of endoplasmic reticulum stress in learning and memory impairment and Alzheimer's disease-like neuropathology in the PS19 and APP^{Swe} mouse models of tauopathy and amyloidosis. *eNeuro* 4, e0025-17
55. Wong, Y. *et al.* (2019) eIF2B activator prevents neurological defects caused by a chronic integrated stress response. *eLife* 8, e42940
56. Rabouw, H.H. *et al.* (2019) The small molecule ISRIB suppresses the integrated stress response within a defined window of activation. *Proc. Natl. Acad. Sci.* 6, 2097–2102
57. Crespillo-Casado, A. *et al.* (2018) A sephin1-insensitive tripartite holophosphatase dephosphorylates translation initiation factor 2α. *J. Biol. Chem.* 293, 7766–7776
58. Antonio, M.D. *et al.* (2013) Resetting translational homeostasis restores myelination in Charcot–Marie–Tooth disease type 1B mice. *J. Exp. Med.* 210, 821–838
59. Wang, L. *et al.* (2014) Guanabenz, which enhances the unfolded protein response, ameliorates mutant SOD1-induced amyotrophic lateral sclerosis. *Neurobiol. Dis.* 71, 317–324
60. Vieira, F.G. *et al.* (2015) Guanabenz treatment accelerates disease in a mutant SOD1 mouse model of ALS. *PLoS One* 10, e0135570
61. Crespillo-Casado, A. *et al.* (2017) PPP1R15A-mediated dephosphorylation of eIF2α is unaffected by sephin1 or guanabenz. *eLife* 6, e26109
62. Colla, E. *et al.* (2012) Endoplasmic reticulum stress is important for the manifestations of α-synucleinopathy in vivo. *J. Neurosci.* 10, 3306–3320
63. Hamamura, K. *et al.* (2014) Attenuation of malignant phenotypes of breast cancer cells through eIF2α-mediated downregulation of Rac1 signaling. *Int. J. Oncol.* 6, 1980–1988
64. Falleta, P. *et al.* (2017) Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes Dev.* 1, 18–33
65. Nguyen, H.G. *et al.* (2018) Development of a stress response therapy targeting aggressive prostate cancer. *Sci. Transl. Med.* 10, eaar2036
66. Lebeaupin, C. *et al.* (2018) Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J. Hepatol.* 69, 927–947
67. Kim, M.J. *et al.* (2012) Attenuation of PERK enhances glucose-stimulated insulin secretion in islets. *Am. J. Physiol. Endocrinol. Metab.* 236, 125–136
68. Gallagher, C.M. *et al.* (2016) Ceapins are a new class of unfolded protein response inhibitors, selectively targeting the ATF6 a branch. *eLife* 5, e11878
69. Gallagher, C.M. and Walter, P. (2016) Ceapins inhibit ATF6 a signaling by selectively preventing transport of ATF6α to the Golgi apparatus during ER stress. *eLife* 5, e11880
70. Plate, L. *et al.* (2016) Small molecule proteostasis regulators that reprogram the ER to reduce extracellular protein aggregation. *eLife* 5, e15550
71. Paxman, R. *et al.* (2018) Pharmacologic ATF6 activating compounds are metabolically activated to selectively modify endoplasmic reticulum proteins. *eLife* 7, e37168
72. Blackwood, E.A. *et al.* (2019) Pharmacologic ATF6 activation confers global protection in widespread disease models by reprogramming cellular proteostasis. *Nat. Commun.* 10, 187
73. Dufey, E. *et al.* (2014) Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 1. An overview. *Am. J. Physiol. Cell Physiol.* 7, 582–594
74. Todd, D.J. *et al.* (2008) The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat. Rev. Immunol.* 8, 663–674
75. Hetz, C. *et al.* (2015) Proteostasis control by the unfolded protein response. *Nat. Cell Biol.* 17, 829–838
76. Martínez, G. *et al.* (2016) Regulation of memory formation by the transcription factor XBP1. *Cell Rep.* 14, 1382–1394
77. Liu, Y. and Gray, N.S. (2006) Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.* 2, 358–364
78. Wang, L. *et al.* (2012) Divergent allosteric control of the IRE1α endoribonuclease using kinase inhibitors. *Nat. Chem. Biol.* 8, 982–989
79. Harrington, P.E. *et al.* (2015) Unfolded protein response in cancer: IRE1α inhibition by selective kinase ligands does not impair tumor cell viability. *ACS Med. Chem. Lett.* 6, 68–72
80. Feldman, H.C. *et al.* (2016) Structural and functional analysis of the allosteric inhibition of IRE1α with ATP-competitive ligands. *ACS Chem.* 11, 2195–2205