

ER stress and the unfolded protein response in neurodegeneration

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Abstract | The clinical manifestation of neurodegenerative diseases is initiated by the selective alteration in the functionality of distinct neuronal populations. The pathology of many neurodegenerative diseases includes accumulation of misfolded proteins in the brain. In physiological conditions, the proteostasis network maintains normal protein folding, trafficking and degradation; alterations in this network — particularly disturbances to the function of endoplasmic reticulum (ER) — are thought to contribute to abnormal protein aggregation. ER stress triggers a signalling reaction known as the unfolded protein response (UPR), which induces adaptive programmes that improve protein folding and promote quality control mechanisms and degradative pathways or can activate apoptosis when damage is irreversible. In this Review, we discuss the latest advances in defining the functional contribution of ER stress to brain diseases, including novel evidence that relates the UPR to synaptic function, which has implications for cognition and memory. A complex concept is emerging wherein the consequences of ER stress can differ drastically depending on the disease context and the UPR signalling pathway that is altered. Strategies to target specific components of the UPR using small molecules and gene therapy are in development, and promise interesting avenues for future interventions to delay or stop neurodegeneration.

Neurodegenerative diseases are characterized by progressive loss of neuronal function in defined regions of the nervous system, culminating in severe dysfunction. These diseases include Alzheimer disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), Huntington disease (HD), prion-related disorders, retinitis pigmentosa and some myelin-related disorders. Each of these conditions has distinct pathophysiological and clinical hallmarks, yet they share a pathological trait: abnormal aggregation of misfolded proteins^{1–3} (BOX 1; FIG. 1). The causal association between accumulation of a specific misfolded protein and the development of pathology in these diseases means that they are often described as proteinopathies, or protein misfolding disorders (PMDs)^{1,4}.

Under physiological conditions, chaperones resident in the cytosol and the endoplasmic reticulum (ER) ensure precise folding of newly synthesized native proteins, and quality control mechanisms identify misfolded proteins and facilitate their degradation via the proteasome, lysosome and autophagy pathways⁵. This process, known as protein homeostasis or proteostasis⁶, is fundamental to the maintenance of cellular health and function, as it prevents abnormal protein aggregation. However, sustaining cellular proteostasis becomes challenging and complex

in PMDs in which misfolded proteins accumulate^{7,8}. One consequence of accumulating misfolded protein is the generation of ER stress^{9,10}, which triggers a rapid and coordinated biochemical response that involves adaptive signalling pathways; this reaction is known as the unfolded protein response (UPR) (FIG. 2). Emerging evidence indicates that ER stress has a vital role in the pathophysiology of PMDs, although the organization, functions and regulation of ER folding and quality control mechanisms are not yet completely understood.

In this Review, we discuss the most recent advances in our understanding of the functional link between ER stress, the UPR, and neurodegeneration, not only in typical PMDs, but also in inflammatory disease and traumatic injury to the nervous system. We provide an in-depth mechanistic explanation of how disease-specific proteins affect ER proteostasis, and discuss novel evidence that links the physiological activity of the UPR with neuronal plasticity and synaptic function. Overall, we bring together emerging evidence that UPR activation occurs in human brain tissue, and analyse evidence from preclinical models that supports functional involvement of ER stress in neurological disease. We also discuss the latest efforts to develop therapeutic strategies for targeting the UPR in neurodegenerative diseases.

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Key points

- Many neurodegenerative diseases involve the accumulation of protein aggregates
- Endoplasmic reticulum (ER) stress triggers activation of the unfolded protein response (UPR), an adaptive reaction that restores cellular protein homeostasis, known as proteostasis
- Dysfunction of proteostasis is associated with abnormal levels of ER stress and is associated with neuronal degeneration in human post-mortem brain tissue
- Targeting the UPR can have distinct and even opposite effects on disease progression, depending on the disease context and the signalling branch that is analysed
- Gene therapy and pharmacological strategies to attenuate ER stress alleviates degeneration in various disease models
- Chronic ER stress not only results in neuronal loss, but also represses the synthesis of synaptic proteins, with implications for cognition and memory, and possibly autism spectrum disorder

Molecular players in UPR signalling

A small fraction of PMDs are familial and involve a genetic mutation that causes misfolding of a specific protein, but in most sporadic cases of the diseases, the same proteins often accumulate and aggregate. This observation has led to the hypothesis that alterations in the buffering capacity of the proteostasis network contribute to the aetiology of PMDs. Importantly, impairment of proteostasis is also exacerbated during ageing^{5,11}, the main risk factor for PMDs (BOX 2).

The function of the ER and the secretory pathway are key elements of the proteostasis network that are altered in brain diseases. The ER is the site of folding for at least one-third of the proteome, a process that is assisted by a complex family of chaperones, foldases, cofactors and enzymes that all mediate various post-translational modifications. Several physiological and pathological conditions that alter the function of the secretory pathway result in ER stress; this perturbation activates the UPR, which controls the stability of RNAs and the rate of protein synthesis, and activates transcription of a large spectrum of genes involved in almost every aspect of the secretory pathway. Typical UPR target genes encode for proteins involved in protein folding, ER-associated degradation (ERAD), vesicular trafficking, autophagy, ER redox control, amino acid metabolism and lipid synthesis⁹. The most studied physiological role of the UPR in tissue homeostasis is its involvement in sustaining the function of specialized secretory cells, in which the pathway is particularly important owing to the high demand in these cells for protein synthesis; this activity creates physiological, rather than pathological, ER stress. Examples of such cells are B cells, endocrine and exocrine pancreatic cells and salivary gland cells¹².

The UPR can be viewed as a simple signal transduction pathway that involves two main components: stress sensors at the ER membrane, and downstream transcription factors that reprogramme gene expression toward stress mitigation or the induction of proapoptotic programmes¹³ (FIG. 3). The UPR target genes vary depending on the tissue context and the type of physiological perturbation that causes ER stress. This variation might result from the formation of different heterodimers

between transcription factors, post-translational modifications and epigenetic changes¹⁴. In mouse and human cells, differences in the patterns of gene expression triggered by ER stress have also been attributed to differences in genetic background^{13,15}. Nevertheless, three main type-I transmembrane proteins initiate the UPR: inositol-requiring protein 1 α (IRE1 α ; a β isoform is selectively expressed in the lung and intestine), activating transcription factor-6 (ATF6, α and β), and protein kinase RNA-like ER kinase (PERK).

Inositol-requiring protein 1

IRE1 α is the most evolutionarily conserved ER stress transducer, and contains RNase and kinase domains within its cytosolic region. Upon activation, IRE1 α dimerizes and autotransphosphorylates, leading to IRE1 α -mediated removal of a 26-nucleotide intron from the mRNA that encodes the transcription factor XBP1 (REF. 16). The consequent shift in reading frame of the XBP1 mRNA results in expression of an active and stable transcription factor called XBP1s. The activity of XBP1s has been linked to prosurvival events that support proteostasis by inducing the expression of various genes that are involved in protein folding and quality-control mechanisms, and by activating the ERAD¹⁴ (FIG. 3). XBP1s also increases biogenesis of the ER and Golgi compartments, thereby increasing the rate of protein secretion. In addition, IRE1 α targets a group of mRNAs and microRNAs (possibly dependent on sequence, secondary structure and/or tissue location) for degradation through a process known as regulated IRE1 α -dependent decay (RIDD)¹⁷. RIDD is thought to have pleiotropic effects in cells, as the phenomenon alters expression of multiple proteins with various functions; these effects include stress mitigation, inflammation and apoptosis¹⁷. Furthermore, active IRE1 α can recruit several adaptor proteins that mediate crosstalk with other stress pathways, including the mitogen-activated protein (MAP) kinase pathway, autophagy, and inflammatory pathways that involve nuclear factor- κ B¹⁴. IRE1 α , therefore, has a dual role in the response to chronic ER stress, mediating adaptation through XBP1s and mediating induction of apoptosis via the MAP kinase pathway and RIDD. On this basis, small molecules that inhibit IRE1 α signalling have been developed¹⁸ and tested in the context of cancer, diabetes mellitus and retinal damage¹⁹, and offer an interesting avenue of intervention in PMDs.

Activating transcription factor-6

Two isoforms of ATF6 — α and β — are expressed ubiquitously in the ER and contain a basic leucine zipper transcription factor domain in its cytosolic domain. ER stress causes ATF6 to translocate to the Golgi apparatus, where endopeptidase S1P and endopeptidase S2P cleave it and release a fragment called ATF6f that includes the transcription factor domain. ATF6f translocates to the nucleus and induces expression of XBP1 and genes that are involved in ERAD and protein folding¹⁶ (FIG. 3). ATF6f cooperates with XBP1s to modulate gene expression under stress.

Autophagy

Self-degradation process with functions that include the removal of misfolded or aggregated proteins and damaged organelles.

Proteostasis

A portmanteau of the words protein and homeostasis, referring to the function of integrated biological pathways within cells that control the biogenesis, folding, trafficking and degradation of proteins present within and outside the cell.

ER stress

A cellular condition that involves accumulation of misfolded and/or unfolded proteins at the ER; ER stress activates the unfolded protein response, which enables adaptation to stress or triggers apoptosis of irreversibly-damaged cells.

Unfolded protein response

A signal transduction pathway that is activated by an accumulation of unfolded or misfolded proteins in the ER lumen; the unfolded protein response mediates adaptation to protein folding stress or the elimination of non-functional cells by apoptosis.

ER-associated degradation

Cellular pathway that targets misfolded proteins at the ER for ubiquitylation and subsequent degradation in the cytosol by the proteasome.

Integrated stress response

An adaptive pathway in eukaryotic cells that is activated by a range of stress conditions that converge on phosphorylation of eukaryotic translation initiation factor 2 α , which leads to a decrease in global protein synthesis and the upregulation of selected genes that promote cellular homeostasis.

Protein disulfide isomerase

One of a family of enzymes in the ER that catalyse the formation, isomerization and breakage of disulfide bonds between cysteine residues within proteins as they fold, enabling the correct arrangement of disulfide bonds in the fully folded state to form quickly.

Protein kinase RNA-like ER kinase

PERK is emerging as a therapeutic target in neurodegenerative diseases, as its signalling pathway is involved in the control of protein synthesis. ER stress triggers the activation of PERK, which directly phosphorylates the protein translation initiation factor eIF2 α , thereby inhibiting protein synthesis and consequently preventing an overload of proteins in the ER lumen¹⁶. In addition, phosphorylation of eIF2 α leads to selective translation of the transcription factor ATF4, which is crucial for the upregulation of genes that encode proteins involved in redox control, amino acid metabolism, autophagy and protein folding and synthesis (FIG. 3). In cells with irreversible damage, ATF4 also engages cell death pathways via induction of the transcription factor DDIT-3 (also known as GADD153, and commonly known as CHOP), reactive oxygen species and members of the apoptosis regulator BCL-2 family¹⁴. The levels of eIF2 α phosphorylation are controlled by serine/threonine-protein phosphatase PP1 (PP1) in complex with either a constitutive regulatory subunit called PPP1R15B or an ER-stress-induced form of the regulatory subunit called GADD34 (also known as PPP1R15A); expression of GADD34 is induced by ER-stress because its encoding gene is activated by CHOP¹⁶. Beyond the UPR, eIF2 α phosphorylation is

also a convergent point of several pathways that make up the so-called integrated stress response, which is triggered by various stimuli, including viral infection, nutrient starvation and haem deficiency²⁰.

Molecular triggers of ER stress

The specific mechanisms that underlie impairment of proteostasis in PMDs have started to be defined by studies that highlight distinct points in the secretory pathway that are disrupted (FIG. 4). For example, in AD, overexpression of β -amyloid precursor in neuronal cell cultures sensitizes cells to ER stress²¹, and many studies (reviewed in REF. 22) have indicated that amyloid- β oligomers, which can accumulate in the ER lumen^{23,24}, cause disruption of ER calcium homeostasis, resulting in a proapoptotic ER stress response. A similar model has been proposed for prion-related disorders^{25–27}. At the molecular level, reduced steady-state levels of calcium in the ER lumen results in suboptimal functioning of calcium-binding chaperones, such as calreticulin, endoplasmic reticulum chaperone (commonly known as GRP94), GRP78 (commonly known as BiP) and protein disulfide isomerase PDI (also known as PDIA1), leading to ER stress²⁸. Current evidence suggests that AD-associated mutations in presenilin-1 and presenilin-2 alter ER calcium homeostasis in the same way, but might also reduce the activity of IRE1 α and expression of BiP, thereby interfering with UPR signalling^{29,30}, although these results are under debate because some studies have indicated that presenilins do not affect ER stress^{31,32}. Accumulation of tau triggers abnormal interactions between ER proteins³³ and essential components of ERAD, thereby impairing this pathway³⁴.

Several molecular mechanisms of proteostasis disruption have been identified in PD. Aggregates of α -synuclein tend to accumulate in the ER lumen and induce ER stress, possibly through an abnormal association with ER chaperones³⁵. Notably, α -synuclein inhibits trafficking of proteins from the ER to the Golgi apparatus, thereby affecting protein maturation and consequently causing ER stress³⁶. Direct impairment of the UPR by α -synuclein has also been proposed, via inhibition of ATF6 activation that results from a physical interaction³⁷. PD-associated mutations in the RING-finger-containing E3 ubiquitin ligase parkin also causes ER stress that results in neuronal degeneration, possibly owing to altered proteasome-mediated degradation^{38,39}. Finally, mutation of the PD-associated gene *ATP13A2* (also known as *PARK9*), which encodes cation-transporting ATPase 13A2, results in misfolding and accumulation of the protein at the ER lumen, triggering chronic activation of the UPR⁴⁰.

In ALS, several independent mechanisms are thought to adversely affect ER physiology. Mutant superoxide dismutase 1 (SOD1, encoded by *SOD1*), physically interacts and inhibits a component of the ERAD pathway known as Derlin-1 (REFS 41,42). Mutant SOD1 has also been observed inside the ER lumen, where it might sequester vital chaperones, such as BiP and PDI^{41,43,44}. Translocation of ALS-associated mutant FUS protein from the nucleus to the cytoplasm in motor neurons is

Box 1 | Protein misfolding associated with neurodegenerative diseases**Alzheimer disease**

- Deposits of intracellular tau aggregate to form neurofibrillary tangles.
- Extracellular aggregates of amyloid- β form amyloid plaques.

Parkinson disease

- Formation of protein inclusion bodies, called Lewy bodies, that contain aggregated α -synuclein and ubiquitin.
- Accumulation of tau deposits.

Huntington disease

- Expansion of the polyglutamine tract in the huntingtin protein causes intracellular aggregation of the protein.

Prion-related disorders (transmissible spongiform encephalopathies)

- Extracellular accumulation of the scrapie form of the prion protein, a pathological isoform of the normal cellular prion protein.

Amyotrophic lateral sclerosis

- Formation of protein inclusions in motor neurons, primarily composed of TDP-43.
- Repeat expansion in *C9orf72* leads to production of dipeptide repeat proteins such as glycine-alanine, which form inclusion bodies.
- Accumulation of mutant superoxide dismutase 1 and TAR DNA binding protein 43 (TDP-43).

Demyelinating disorders

- Accumulation of mutant myelin proteins at the endoplasmic reticulum in Schwann cells and oligodendrocytes.

Frontotemporal dementia

- Accumulation of repeat-associated non-ATG translation peptide products, TDP-43 and tau inclusions.

Retinitis pigmentosa

- Accumulation of misfolded mutant rhodopsin inside the endoplasmic reticulum of retinal cells.

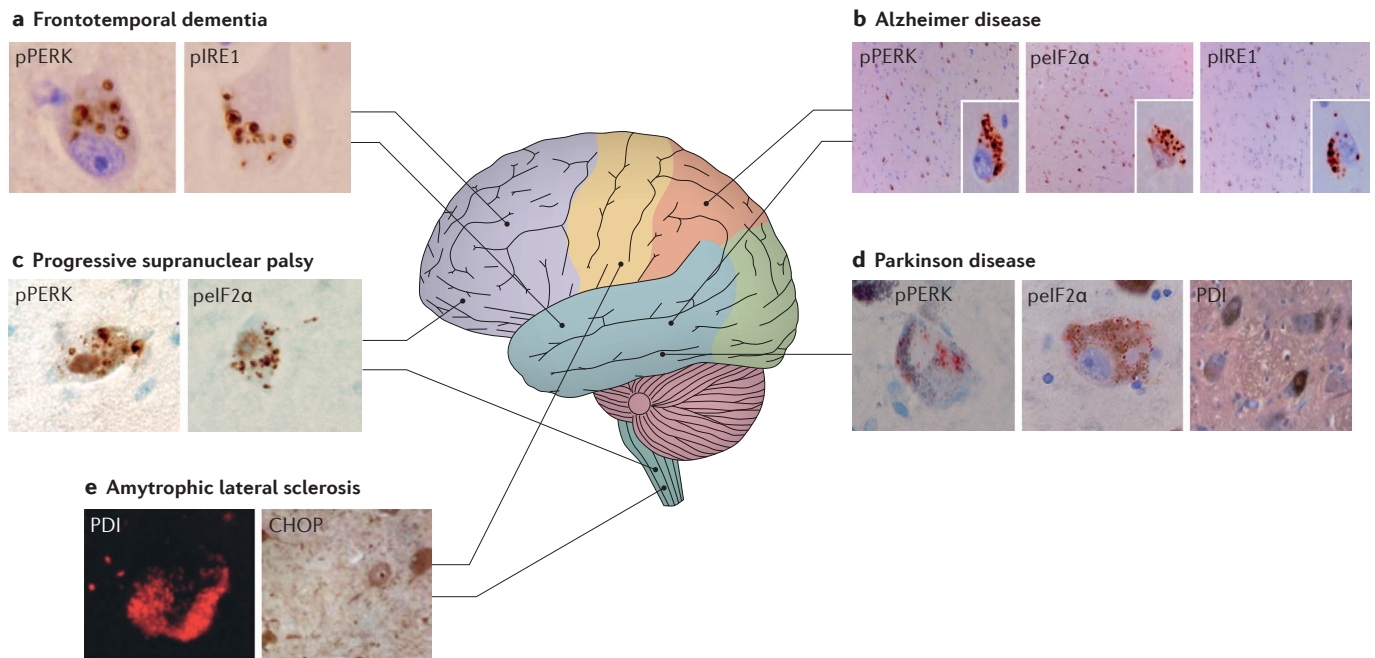


Figure 1 | Protein aggregates in tissue from patients with neurodegenerative disease. Most neurodegenerative diseases have distinct clinical manifestations, but they share accumulation of protein aggregates and inclusions that contain specific proteins in distinct brain regions; these aggregates are associated with an endoplasmic reticulum stress reaction. PDI, protein disulfide isomerase; p, phosphorylated; PERK, protein kinase RNA-like ER kinase; IRE1, inositol-requiring protein 1. Part **a** reproduced with permission from John Wiley and Sons © Nijholt, D. A. *et al. J. Pathol.* **226**, 693–702 (2012). Part **b** reproduced with permission from Elsevier © Jeroen, J. M. *et al. Am. J. Pathol.* **174**, 1241–1251 (2009). Part **c** reproduced with permission from BioMed Central © Stutzbach, L. D. *et al. Acta Neuropathol. Commun.* **1**, 31 (2013). Part **d** reproduced with permission from Elsevier © Hoozemans, J. J. *et al. Biochem. Biophys. Res. Commun.* **354**, 707–711 (2007) (left and middle panels) and © Conn, K. J. *et al. Brain Res.* **1022**, 164–172 (2004) (right panel). Part **e** reproduced with permission from Elsevier © Atkin, J. D. *et al. Neurobiol. Dis.* **30**, 400–407 (2008) (left panel) and © Ito, Y. *et al. Neurobiol. Dis.* **36**, 470–476 (2009) (right panel).

associated with ER stress and fragmentation of the Golgi apparatus⁴⁵. FUS also interacts with PDI upon induction of ER stress⁴⁶, and the function of PDI is also affected by nitrosylation of the active site, which has been reported in ALS⁴⁷, PD and AD⁴⁸. ALS-linked mutant vesicle-associated protein-associated protein B (VAPB) can interact with ATF6 and XBP1, altering their activities and subcellular distribution⁴⁹. In patients with a *C9orf72* repeat expansion, the glycine–alanine dipeptide repeat protein causes neurotoxicity by initiating ER stress and altering the ubiquitin-proteasome system through an unknown mechanism⁵⁰.

Two studies have shown that mutant huntingtin impairs ERAD through abnormal protein–protein interactions^{51,52}. In addition, fragments of huntingtin other than the polyglutamine tract can alter the morphology of the ER through an aberrant interaction with dynamin-1, resulting in ER dilatation and chronic stress⁵³. Perturbations to many other molecular components of the secretory pathway have been associated with ER stress in PMDs^{7,33,54}. Overall, the common alterations to ER proteostasis that are observed in neurodegenerative diseases highlight disruption of ER calcium homeostasis, vesicular trafficking, ERAD activity and UPR dysregulation, and abnormal ER chaperone function.

ER stress in human neurodegeneration

Most studies of the UPR in humans have been performed with autopsy brain samples (FIG. 1). Several neurodegenerative disorders have also been modelled with human induced pluripotent stem cells (iPSCs). Patient-specific iPSC-derived neuronal cells largely recapitulate relevant disease phenotypes, enabling the characterization of disease mechanisms and the screening for novel therapeutic targets. Across various PMDs, multiple studies of human tissue and iPSCs have demonstrated activation of the UPR. This consistency suggests that ER stress has a central and conserved role in the pathogenic neuronal response.

Studies of human tissue

In brain tissue from patients with AD, increases in UPR activation markers are widespread and occur early relative to markers in control brain tissue from people without dementia. For example, increased expression levels of the ER chaperone BiP) have also been observed in regions affected by AD, such as the hippocampus and temporal cortex^{55,56}. Furthermore, augmented PERK and IRE1α signalling has been observed in AD neurons that contain abnormally phosphorylated tau^{55–58}. Remarkably, levels of IRE1α phosphorylation directly correlated with the Braak stage of pathology in patients with AD⁵⁹.

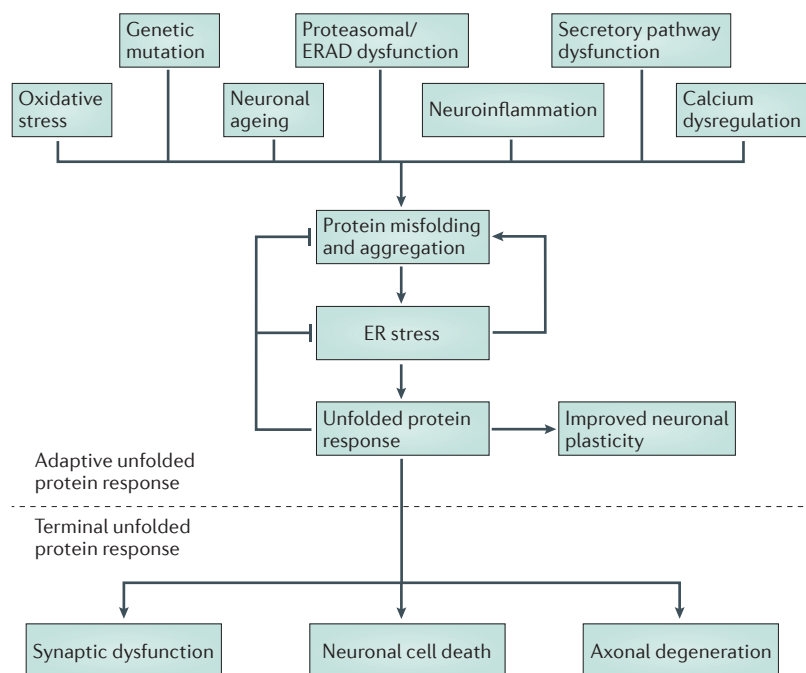


Figure 2 | ER stress and proteostasis in neurodegenerative diseases. Ageing, environmental factors and mutation of specific disease-related genes can trigger misfolding of a particular protein, leading to formation of aggregates that range from small oligomeric species to large inclusion bodies. This abnormal aggregation results in endoplasmic reticulum (ER) stress. ER stress can increase aggregation of disease-related proteins via a feedback loop by altering the folding and quality-control capacity of the cell or by altering the expression of disease-related genes. ER stress engages unfolded protein response (UPR) sensors that activate distinct downstream responses to improve protein folding and quality-control mechanisms to reduce ER stress. Long-term ER stress over-rides the adaptive responses of the UPR, and induces apoptosis. Chronic inhibition of protein synthesis can also reduce synthesis of synaptic proteins, thereby impairing neuronal function. ERAD, ER-associated degradation.

Increased UPR activation has also been reported in other tauopathies, such as progressive supranuclear palsy, Pick disease, familial FTD with parkinsonism linked to chromosome 17 (REF. 60), and frontotemporal lobar degeneration with tau pathology⁶⁰. In PD, UPR activation has been observed early in the disease, associated with increased phosphorylation of PERK and eIF2 α in neuromelanin-positive neurons of the substantia nigra^{61,62}. Similarly, increased levels of UPR markers have been observed in multiple system atrophy, a disease that, like PD, is associated with accumulation of α -synuclein⁶³.

In ALS, expression of components of all three UPR pathways is increased in the spinal cord of patients with sporadic and familial forms of the disease^{64–68}. Moreover, PDI is upregulated in the cerebrospinal fluid of patients with sporadic ALS, and its expression is induced in motor neurons of these patients, indicating that PDI dysfunction contributes to the ALS pathology and might aid diagnosis⁶⁴. Proteomic screening for biomarkers in the blood of patients with sporadic ALS also identified that the major proteins upregulated were a cluster of ER-stress-induced chaperones⁶⁹. Global gene expression profiling of brain tissue from patients with ALS

associated with a *C9orf72* repeat expansion revealed that UPR alterations are a major signature of the pathology in cerebellum⁷⁰.

In prion-related disorders, such as Creutzfeldt–Jakob disease (CJD), elevated levels of ER stress markers, including protein disulfide isomerase A3 (commonly known as ERp57 and also known as GRP58), BiP and GRP94, have been observed in cortical samples from patients with sporadic forms of CJD and in those with infectious forms of the disease²⁵. The main upregulated protein that was identified in a proteomic screening study in patients with CJD was ERp57 (REF. 71). However, monitoring of PERK and IRE1 α phosphorylation in other studies failed to detect signs of ER stress in CJD^{58,72}.

In post-mortem cortex tissue from patients who had HD, increased transcript levels of the UPR effectors BiP, CHOP and the ERAD component HERP have been observed⁷³. Analysis of global gene expression data has also revealed major alterations in UPR signalling in HD⁷⁴, and increased splicing of XBP1 has been observed in the striatum of patients with HD⁷⁵.

Studies of iPSC-derived human neurons

Analysis of cortical neurons that were generated from iPSCs from patients with PD caused by α -synuclein mutations revealed strong and early ER stress that led to disruption of proteostasis accompanied by nitrosative stress and impairment of ERAD⁷⁶. Similarly, levels of UPR markers were elevated in dopaminergic neurons that were generated from iPSCs from patients with PD who had α -synuclein and glucocerebrosidase mutations⁷⁷. In ALS, transcriptional analysis of motor neurons derived from iPSCs from patients carrying a *SOD1* mutation demonstrated that ER stress was high in these cells. Remarkably, UPR alterations observed in motor neurons with *C9orf72* repeat expansions were similar to those in neurons with *SOD1* mutations, highlighting the susceptibility of motor neurons to ER stress⁷⁸. Another study also indicated that decreased survival of iPSC-derived motor neurons with *C9orf72* repeat expansions can be attributed to ER stress in combination with impaired calcium homeostasis and abnormal mitochondrial function⁷⁹. One study has also shown that motor neuron cultures generated from iPSCs from patients with ALS-associated *VAPB* mutations develop chronic ER stress, resulting in secondary oxidative stress, mitochondrial dysfunction, synaptic loss and cell death⁸⁰.

iPSC modelling has also provided some insight into other PMDs. In HD, analysis of iPSCs derived from patients has revealed ER calcium dyshomeostasis, which could affect protein folding at the ER⁸¹. ER stress has also been identified as a salient feature in familial AD and retinitis pigmentosa through the use of iPSC modelling^{82–84}.

Linking ER stress to neurodegeneration

Chronic activation of the UPR has emerged as a conserved feature among various neurodegenerative diseases on the basis of animal models and post-mortem studies of tissue from patients. Selective neuronal populations seem to be specifically vulnerable to ER stress⁸⁵,

Box 2 | ER proteostasis and ageing

Perturbed neuronal proteostasis is a salient feature of ageing as well as of protein misfolding disorders¹¹. A reduction in the buffering capacity of the proteostasis network during ageing might increase the risk of neurodegeneration by increasing the accumulation of abnormal protein aggregates. Protein aggregates might also propagate via a prion-like mechanism, thereby spreading pathology through the brain¹⁸⁰. Studies in model organisms indicate that the neuronal unfolded protein response (UPR) has a central role in controlling global proteostasis. For example, a study conducted with *Caenorhabditis elegans* suggest that expression of the ER-stress-induced transcription factor XBP1s in neurons activates global responses in peripheral tissues that improve proteostasis and age resilience^{11,188}. Experiments in mouse models have also indicated that XBP1s expression in the hypothalamus promotes cell-nonautonomous UPR activity in the periphery, including the liver, with effects on global energy metabolism¹⁸⁹.

and studies in mice and humans have shown that ER stress and an impaired UPR are directly associated with neurodegeneration⁸⁶. Over the past decade, genetic and pharmacological manipulation of the UPR have been used to understand the causal link between ER stress and neurodegeneration (FIG. 3). These studies have exposed a multifaceted scenario wherein distinct signalling components of the UPR have specific, and sometimes even opposite, effects on the disease pathophysiology depending on the disease type, the neurons affected and the stage of the disease (TABLE 1). In this section, we discuss the outcomes of key studies that have addressed this functional link, focusing on *in vivo* models and their use for defining optimal targets for disease intervention.

PERK signalling

Generation of small molecules and genetic tools that enable manipulation of PERK signalling in a disease-specific manner in the past 5 years has transformed the study of ER stress and neurodegeneration (TABLE 1). The therapeutic value of PERK modulation has been demonstrated by successful use of an orally administered PERK inhibitor (GSK2606414) in mice⁸⁷, and the successful use of several eIF2 α phosphatase inhibitors, including salubrinal⁸⁸ (which blocks PPP1R15B and PPP1R15A), guanabenz⁸⁹ and its derivative sephin-1 (REF. 90) (which inhibit PPP1R15A). Similarly, a small molecule called ISRIB (integrated stress response inhibitor) efficiently blocks the consequences of eIF2 α phosphorylation^{91,92}.

Initial studies indicated that *Perk* haploinsufficiency accelerates experimental ALS in transgenic mice with a *SOD1* mutation⁹³. Consistent with this observation, genetic ablation of GADD34 (REF. 94) or the administration of the eIF2 α phosphatase inhibitors guanabenz, sephin-1 or salubrinal^{90,95–97} — thereby enhancing blockage of protein translation — had positive effects in improving motor neuron survival and motor performance and delaying the death of the animals. Similarly, eIF2 α phosphatase inhibitors reduced neurodegeneration in zebrafish and *Caenorhabditis elegans* models of TDP-43 pathology⁹⁸, although another mouse study showed that treatment with guanabenz could accelerate progression of experimental ALS⁹⁹. Ablation of ATF4 expression in mice with *SOD1* mutations protects against ALS, possibly by reducing the levels of apoptosis components, including

CHOP¹⁰⁰. These observations suggest that PERK signalling has a dual role in ALS, promoting survival by repressing protein synthesis upon ER stress, but promoting apoptosis if the stress becomes chronic and irreversible.

A pathological role of ATF4 has been reported in AD. Studies in mouse models and cell culture have shown that local expression of ATF4 in axons leads to transmission of neurodegenerative signals through cell-nonautonomous mechanisms¹⁰¹. In experimental PD, *Chop* deficiency was protective¹⁰² and eIF2 α phosphatase inhibition with salubrinal alleviated symptoms, but neither affected survival of dopaminergic neurons¹⁰³.

Some evidence suggests that ER stress contributes to neurodegeneration in diseases that involve mutation of myelin proteins that causes their misfolding and accumulation at the ER. For example, a pathogenic role of PERK has been demonstrated in mouse models of Charcot-Marie-Tooth disease, a group of inherited disorders that affect the peripheral nerves. Several reports have systematically dissected the effect of PERK signalling on Schwann cell survival and motor deficits; these studies have used *Perk*, *Chop* and *Gadd34* knockout mice, and treatment with salubrinal, and have revealed global neuroprotection and improved motor recovery^{104–106} (TABLE 1). Treatment with sephin-1 prevented the development of experimental Charcot-Marie-Tooth disease⁹⁰; one study has brought into question the specificity of guanabenz and its derivative sephin-1 for inhibition of the eIF2 α phosphatase subunit PPP1R15A¹⁰⁷, but their actions are neuroprotective regardless of their target. In other demyelinating diseases that affect oligodendrocyte function, such as Pelizaeus-Mezbarcher disease, *Chop* deficiency in mice surprisingly exacerbated the pathology¹⁰⁸, but the mechanism of action was not defined.

IRE1 α and XBP1

The role of IRE1 α signalling on neurodegeneration has been studied extensively in the context of downstream XBP1 function (TABLE 1), and most studies suggest that XBP1s has a neuroprotective role. Activity of the XBP1 transcription factor has been widely associated with adaptive programmes that alleviate ER stress, so gene therapy in which XBP1s (the active form) is delivered has been tested as a strategy to artificially activate the pro-survival UPR. Delivery of recombinant adeno-associated viruses (AAVs) to express XBP1s in selective brain regions provides outstanding neuroprotection in various mouse models of PMDs (reviewed in REF. 109). For example, local injection of viruses to express XBP1s in the substantia nigra prevented degeneration triggered by PD-inducing neurotoxins^{110,111}. Similarly, expression of XBP1s reduced aggregation of mutant huntingtin in the striatum¹¹² in a mouse model of HD.

Genetic ablation of *Xbp1* specifically in the nervous system of mouse models of ALS and HD has produced surprising observations. Despite the expectation that targeting this major pro-survival mediator of the UPR will accelerate disease progression, conditional genetic deletion of *Xbp1* in the CNS protected against experimental ALS⁶⁵. XBP1 deficiency reduced motor neuron loss and aggregation of mutant SOD1, thereby delaying

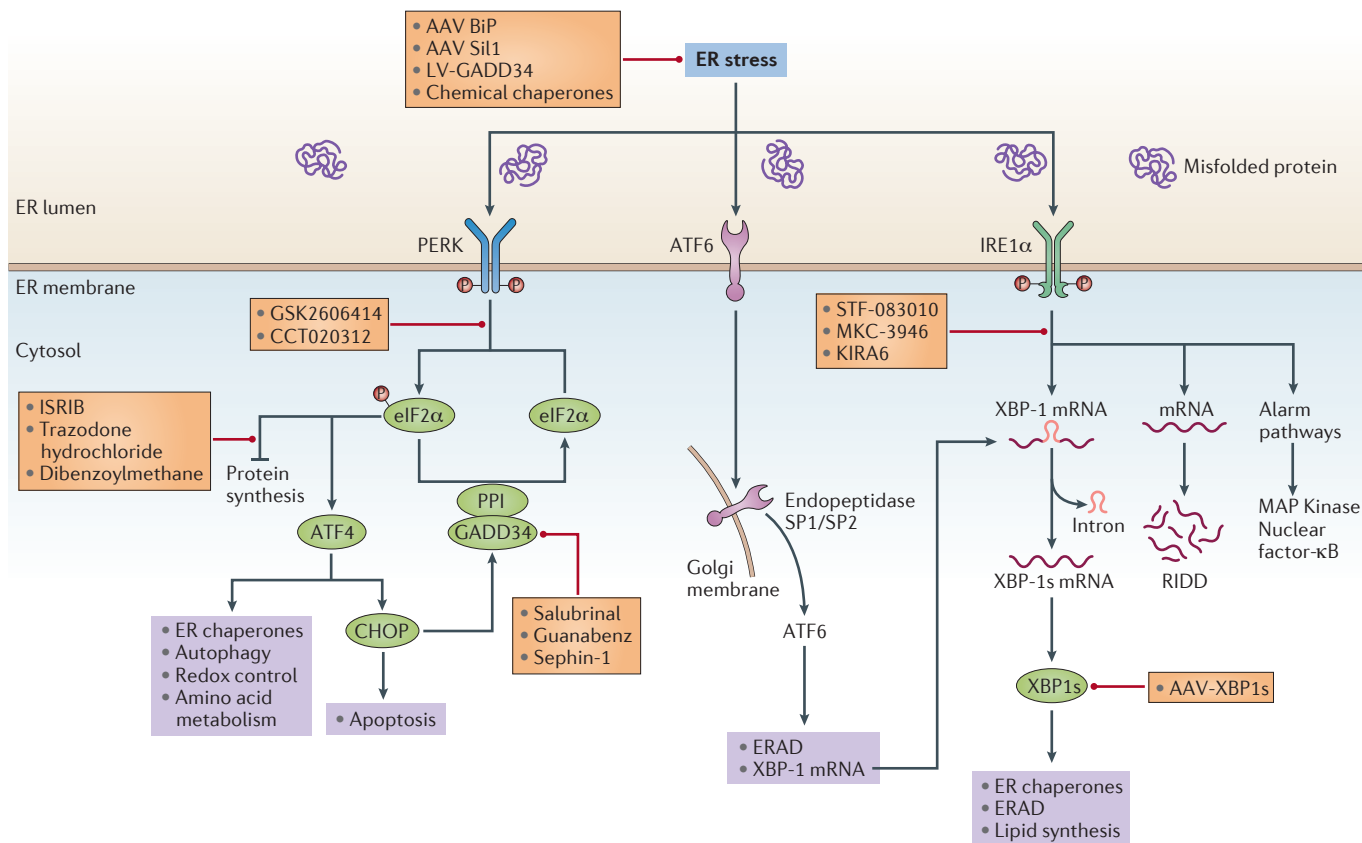


Figure 3 | Unfolded protein response pathways and interventions. Accumulation of misfolded proteins in the endoplasmic reticulum (ER) activates the unfolded protein response sensors protein kinase RNA-like ER kinase (PERK), activating transcription factor (ATF) 6 and IRE (inositol-requiring protein) 1 α . PERK activation leads to phosphorylation of eIF2 α and consequent inhibition of protein translation and expression of the transcription factor ATF4. ATF4 translocates to the nucleus and induces expression of pro-survival genes. ATF4 also controls genes related to apoptosis, including CHOP. CHOP in turn can induce the expression of GADD34, promoting the dephosphorylation of eIF2 α under prolonged ER stress. Upon ER stress, ATF6 is transported to the Golgi apparatus, where it is cleaved by endopeptidase S1P and endopeptidase S2P, thereby releasing the cytosolic ATF6 fragment (ATF6f) that operates as a transcription factor. ATF6f induces genes required for ER-associated degradation and modulates XBP1 mRNA levels. Active IRE1 α induces splicing of mRNA that encodes XBP1, leading to expression of the active transcription factor XBP1s that upregulates pro-survival processes. IRE1 α also associates with the adaptor protein TRAF2 (TNF receptor-associated factor 2) and induces mitogen-activating protein (MAP) kinase activation that modulates autophagy and apoptosis. IRE1 α activity also induces regulated IRE1 α -dependent mRNA decay (RIDD), which affects various pathways, including lipid biosynthesis, microRNAs, inflammation and apoptosis. Attenuation of ER stress in disease can be achieved by intervention at various points, shown in red using pharmacological or gene therapy approaches. AAV, adeno-associated virus; ERAD, ER-associated degradation; ISIRIB, integrated stress response inhibitor; KIRA6, IRE1 α kinase inhibiting RNase attenuator 6; LV, lentivirus; PPI, protein phosphatase 1.

disease onset and death. These beneficial effects were associated with increased autophagy in motor neurons that increased mutant SOD1 clearance⁶⁵. Results of *Xbp1* ablation in a mouse experimental model of HD were almost identical⁷⁵, suggesting a tight homeostatic balance between the UPR and autophagy that prevents neurodegeneration. Another report suggested that sustained activation of IRE1 α in HD can trigger neuronal loss¹¹³. By contrast, blocking of XBP1 expression did not modify prion pathogenesis *in vivo*¹¹⁴.

Studies in fly models also indicate that XBP1s overexpression protects neurons against amyloid- β and tau toxicity^{115,116}. In agreement with this concept, targeting of IRE1 α expression in the brain in a mouse model of AD indicated a pathological role of the pathway: ablation of IRE1 α reduced amyloid- β deposition

and fully restored synaptic and cognitive function⁵⁹. These beneficial effects were mapped to the control of APP protein stability by XBP1s, which accelerated the amyloid cascade in the disease model. Studies in *C. elegans* also showed that knocking down XBP1 protects against amyloid- β toxicity, thereby increasing lifespan, via a mechanism that might involve hyperactivation of IRE1 α , which controls autophagy through the MAP kinase pathway¹¹⁷. These studies are in line with other studies in models of diabetes mellitus and retinal degeneration in which overactivation of IRE1 α is pathogenic¹¹⁸.

In general, XBP1 deficiency in the mouse brain does not result in evidence of ER stress or other conserved molecular alterations to compensate for the absence of XBP1 via the activation of the PERK branch, but the

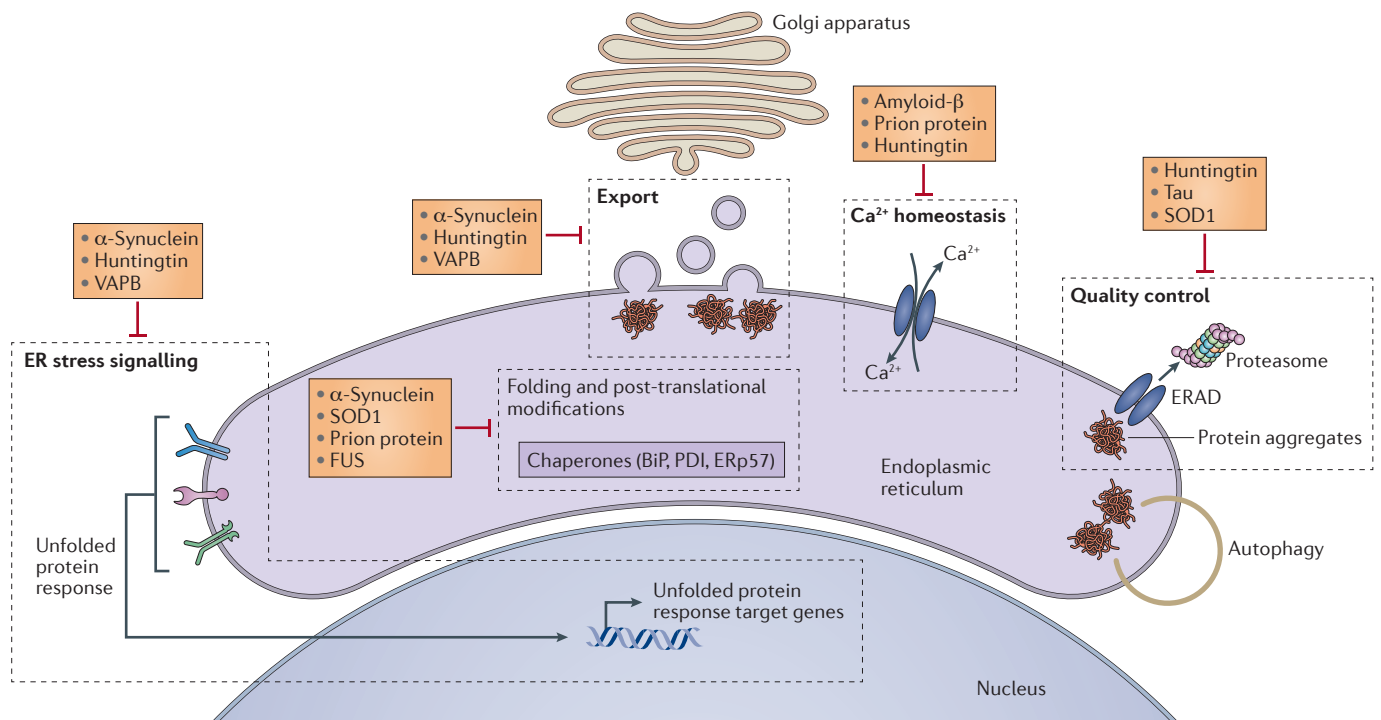


Figure 4 | Mechanisms that trigger ER stress in neurodegenerative disease. Correctly folded proteins are processed in the endoplasmic reticulum (ER) and trafficked to the Golgi apparatus for further maturation and distribution to their final destination. Protein folding and maturation at the ER is altered in neurodegenerative disease owing to the effects of protein aggregates on various mechanisms, which include inhibition of protein folding by inhibiting chaperones, interference with the ER-associated degradation pathway, perturbation of ER-to-Golgi trafficking, inhibition of proximal unfolded protein response components, and exacerbation of ER calcium release. ERAD, ER-associated degradation; FUS, fused in sarcoma; PDI, protein disulfide isomerase; SOD1, superoxide dismutase 1; VAPB, vesicle-associated membrane protein-associated protein B.

same is not the true in the substantia nigra. Knockdown of XBP1 in the adult mouse substantia nigra results in chronic ER stress, triggering degeneration of dopaminergic neurons¹¹⁰. When XBP1 expression is ablated during brain development, only mild (non-toxic) ER stress occurs in the substantia nigra, but this stress protects from PD via upregulation of the adaptive UPR (upregulation of ER chaperones and autophagy)^{110,119}. In agreement with these observations, studies in fly and rat models of PD indicate that treatment with non-toxic doses of tunicamycin, which induces adaptive ER stress signals, also attenuates experimental PD in this way, a mechanism of protection known as ER hormesis¹²⁰.

ATF6 signalling

Involvement of ATF6 in neurodegeneration has been poorly studied (TABLE 1). Some studies have been performed in animal models of PD, and have shown that mice with a deficiency of ATF6α are hypersensitive to PD-inducing neurotoxins^{121,122}. ATF6α knockout mice exhibit reduced basal levels of BiP in dopaminergic neurons¹²², suggesting that activity of the ATF6 pathway is required to maintain proteostasis in this neuronal population. The function of ATF6 is repressed in models of HD, and this repression contributes to disease pathogenesis^{123,124}. ATF6α and ATF6β have complementary and redundant activities¹²⁵, so the role of this specific

branch of the UPR requires further investigation. Given the results of these studies, gain-of-function approaches that involve delivery of ATF6f via gene therapy should be used to define the therapeutic value of targeting this UPR signalling branch for disease treatment.

ER chaperones

UPR target genes whose protein products are chaperones and cofactors involved in protein folding and quality control are also emerging as relevant players in neurodegeneration. One example of these genes is *SIL1*, which encodes an adenine nucleotide exchange factor for BiP. Mutation of *SIL1* causes neurodegeneration associated with abnormal protein aggregation and cerebellar degeneration¹²⁶. *SIL1* mutations have also been linked to Marinesco–Sjögren syndrome, a condition that causes cerebellar ataxia, mental retardation and muscle weakness. Another study showed that the expression level of *SIL1* determines motor neuron susceptibility to ER stress¹²⁷. Gene therapy to increase expression of *SIL1* in the nervous system delayed development of pathology in experimental ALS¹²⁷. Genetic inactivation of BiP itself results in spontaneous degeneration and motor alterations during ageing in association with aggregation of wild-type SOD1 in mice (REF. 128). Interestingly, promoter polymorphisms in the ER chaperones BiP and GRP94 have also been genetically linked

Hormesis

A phenomenon in which an agent that is toxic to a biological system at high doses has beneficial effects on that system at lower doses.

Table 1 | Functional impact of ER stress in neurodegenerative diseases

Disease	Model	UPR manipulation	Phenotype	Refs
PERK signalling				
Amyotrophic lateral sclerosis	Mutant SOD1 Tg mice	<i>EIF2AK3</i> ^{+/-}	Disease exacerbation, enhanced SOD1 aggregation	93
		<i>PPP1R15A</i> ^{+/-}	Disease exacerbation, enhanced SOD1 aggregation	94
		Salubrinal	Extended life span	97
		Guanabenz	Delayed disease progression, extended life span	95,96
		Guanabenz	Exacerbated disease	99
		Sephin-1	Neuroprotection, delayed disease onset	90
		<i>ATF4</i> KO	Protection against disease progression	100
Alzheimer disease	PSEN1/APP mice	<i>EIF2AK3</i> cKO	Improved learning and memory and LTP	152,153
		siRNA <i>ATF4</i>	Neuroprotection	101
	APP mice	ISRIB	No improvement in learning and memory	154
	Tau Tg mice	GSK2606414	Global neuroprotection	145
		CCT020312	Global neuroprotection	150
		Trazodone	Neuroprotection, reduced tau phosphorylation	148
		Dibenzoylmethane	Neuroprotection	148
Charcot–Marie–Tooth disease	Mutant P-myelin mice	Salubrinal	Neuroprotection, Schwann cell survival, motor recovery	106
		<i>CHOP</i> KO	Global neuroprotection	104
		<i>PPP1R15A</i> KO	Global neuroprotection	105
		<i>EIF2AK3</i> cKO	Global neuroprotection	106
		Sephin-1	Improved motor function and neuroprotection	90
Huntington disease	Mutant Htt Tg mice	<i>ATF4</i> KO	No effect on mutant Htt aggregation	75
Parkinson disease	α -synuclein Tg mice	Salubrinal	Neuroprotection	103
	Neurotoxins	<i>CHOP</i> KO	Neuroprotection	102
Pelizaeus–Merzbacher disease	Proteolipid mutant mice	<i>CHOP</i> KO	Disease exacerbation, oligodendrocyte apoptosis	108
Prion-related disease	Scrapie prion infected mice	Salubrinal	Disease exacerbation	143
		GSK2606414	Reduced neurodegeneration, delayed disease progression	144
		ISRIB	Delayed onset, improved survival	146
		LV-GADD34	Delayed onset	143
		Trazodone	Delayed onset	148
		Dibenzoylmethane	Delayed onset	148
IRE1 signalling				
Amyotrophic lateral sclerosis	Mutant SOD1 Tg mice	<i>XBP1</i> cKO	Neuroprotection, reduced SOD1 aggregation	65
Alzheimer disease	APP/PSEN1 mice	<i>IRE1</i> cKO	Reduced A β load, synaptic function, cognition and glial activation	115,116
		AAV <i>XBP1s</i>	Improved neuronal plasticity and behaviour	162
Huntington disease	Mutant Htt Tg mice	<i>XBP1</i> cKO	Improved motor performance, reduced Htt	75
		AAV <i>XBP1s</i>	Improved motor performance, reduced Htt	112
Parkinson disease	Neurotoxins	AAV <i>XBP1s</i>	Reduced dopaminergic neuron loss	110
		AV <i>XBP1s</i>	Reduced dopaminergic neuron loss	111
Prion-related disease	Scrapie prion infected mice	<i>XBP1</i> cKO	No effect on disease progression or prion replication	114
ATF6 signalling				
Parkinson disease	Neurotoxins	<i>ATF6</i> KO	Enhanced neurodegeneration	121,122

AV, adenovirus; AAV, adeno-associated virus; ATF4, activating transcription factor 4; cKO, conditional knockout; ER, endoplasmic reticulum; Htt, huntingtin protein; IRE1, inositol-requiring protein 1; ISRIB, integrated stress response inhibitor; KO, knockout; LV, lentivirus; LTP, long-term potentiation; PERK, protein kinase RNA-like ER kinase; PSEN1, presenilin 1; siRNA, small interfering RNA; SOD1, superoxide dismutase; Tg, transgenic; UPR, unfolded protein response.

to the development of bipolar disorders in humans^{129,130}. Overall, these studies suggest that disruption of ER proteostasis can result in severe neuronal dysfunction.

One major folding and quality control pathway for glycoproteins in the ER is mediated by calnexin and calreticulin, lectin chaperones that form a complex with ERp57, which assists with disulfide bond formation. Genetic ablation of calnexin in mice leads to severe demyelination, resulting in reduced axonal conduction velocity and consequent motor defects^{131,132}. Similarly, genetic ablation of calreticulin accelerates muscle denervation in ALS¹³³. Mutations in the genes encoding PDI and ERp57 have been proposed as risk factors for ALS^{134–136}. Conditional deletion of ERp57 in the nervous system triggers motor neuron dysfunction associated with an abnormal structure of neuromuscular junctions and reduced expression of certain synaptic proteins, such as synaptic vesicle protein 2 (REF. 137). Expression of ALS-associated mutant forms of PDI and ERp57 in zebrafish models dramatically alters the morphology of the neuromuscular junction and causes motor deficits¹³⁷. Mutations in other genes of the proteostasis network that are involved in proteasome degradation, such as *UBQLN2*, which encodes ubiquilin 2, and *UCHL1*, which encodes ubiquitin *c*-terminal hydrolase L1, are also associated with ALS, and their expression in transgenic mice results in ER stress and motor dysfunction^{138,139}. All of these studies suggest that motor neurons are highly susceptible to perturbations in ER proteostasis. However, in transgenic mice that overexpressed ERp57 in the nervous system, peripheral nerve degeneration was delayed, although overexpression of ERp57 in a mouse model of PD did not influence dopaminergic neuron survival¹⁴⁰.

Gene therapy and pharmacological strategies have been developed to improve protein folding and reduce pathological levels of ER stress. Delivery of BiP-overexpressing AAVs to the substantia nigra of rats delayed the progression of PD that is triggered by α -synuclein overexpression, and improved motor performance and dopaminergic neuron survival¹⁴¹. The same approach had outstanding effects in a model of retinitis pigmentosa, in which sub-retinal delivery of AAV-BiP diminished photoreceptor apoptosis, attenuated ER stress levels and improved visual function in transgenic rats with mutant rhodopsin¹⁴². Another therapeutic approach that has been used is administration of chemical chaperones, which are low-molecular-weight compounds that stabilize the protein structure and buffer abnormal protein aggregation. The best characterized chemical chaperones in a disease context are 4-phenyl butyrate (4-PBA), tauroursodeoxycholic acid (TUDCA), and the sugar trehalose, which are all FDA-approved and have good safety profiles in humans. Treatment with various chemical chaperones in mouse models of ALS, HD and PD have indicated that they provide considerable neuroprotection (reviewed in REF. 19), although in most of these studies, ER stress levels were not determined. In summary, the evidence suggests that alterations to the ER folding machinery underlie neuronal dysfunction in various neurodegenerative diseases, indicating that strategies to improve folding could have important therapeutic effects.

Synaptic function and plasticity

Novel evidence suggests that PERK and IRE1 α -XBP1 signalling are relevant to neurodegenerative diseases not only because they mediate the attenuation of chronic ER stress, but also because they influence synaptic function through novel mechanisms. In combination, the findings, which are described in the next section, suggest that these pathways alter neuronal function by modulating the expression of important synaptic proteins. Effects of UPR signalling on the establishment of synapses and neuronal plasticity have implications for cognitive and memory function in the context of PMDs.

PERK and synaptic function

Beyond its role as a mediator of ER stress in all cells, evidence suggests that PERK signalling has an additional role in neuronal physiology. Chronic PERK signalling is thought to repress the expression of a cluster of synaptic proteins, leading to altered neuronal plasticity and behaviour, and several studies have indicated that this effect is relevant in neurodegenerative disease.

In prion-infected mice, functional studies have demonstrated that sustained phosphorylation of eIF2 α dramatically reduces expression of synaptic proteins¹⁴³. Oral administration of PERK inhibitors to prion-infected animals restored levels of synaptic proteins and delayed the disease course¹⁴⁴. However, unexpectedly, PERK inhibition did not affect the levels of prion misfolding and aggregation.

In mouse models of tau-mediated FTD, the PERK inhibitor GSK2606414 provided neuroprotection associated with improved synaptic function and neuronal survival that was associated with reduced tau phosphorylation¹⁴⁵. However, GSK2606414 was associated with adverse effects on pancreatic function owing to a high toxicity of this compound to β cells¹⁴⁶. Furthermore, the specificity of GSK2606414 has been questioned, as it blocks receptor-interacting serine–threonine protein kinase 1 (RIPK1), a central component of the necroptosis machinery¹⁴⁷. These adverse effects were not observed when ISRIB was tested to target the pathway in prion-infected animals¹⁴⁶. A 2017 drug screen identified that two FDA-approved drugs — trazodone hydrochloride and dibenzoylmethane — are de-repressors of translational attenuation mediated by phosphorylated eIF2 α ¹⁴⁸. Use of these compounds in mouse models of prion-related disorders and FTD protected against neurodegeneration, and no toxicity was associated with use of clinically-relevant concentrations¹⁴⁸.

In contrast to these studies, another report suggested that PERK signalling is beneficial in FTD. Pharmacological activation of PERK signalling with the drug CCT020312 (REF. 149) improved dendritic spine density in a mouse model of tau-mediated FTD, and this protection was associated with improved cognitive and motor function and attenuated tau pathology. These effects were associated with the phosphorylation of a different PERK substrate, nuclear factor erythroid 2-related factor 2, which is a central component of the antioxidant response¹⁵⁰. This observation is consistent with the fact that patients with Wolcott–Rallison syndrome,

a rare condition in which diabetes mellitus is caused by loss-of-function mutations in *EIF2AK3*, which encodes PERK, develop early signs of neurodegeneration, including the accumulation of neurofibrillary tangles¹⁵¹.

In AD, chronic PERK signalling has adverse effects on synaptic function. In mouse models of AD, ablation of PERK improved memory deficits and long-term potentiation^{152,153}, although inhibition of the integrated stress response with ISRIB (a negative regulator of neuronal plasticity) did not rescue memory deficits in transgenic AD mice¹⁵⁴. Deletion of PERK in the adult mouse forebrain recapitulates multiple behavioural phenotypes associated with impaired cognition and information processing¹⁵⁵. However, only in the hippocampus did PERK signalling improve synaptic function by suppressing long-term depression¹⁵⁶. Overall, many studies indicate that the integrated stress response operates as a global negative regulator of synaptic plasticity through the phosphorylation of eIF2 α at basal levels (reviewed in REF. 157).

***IRE1 α* and *XBP1* in synaptic function**

Studies have suggested that XBP1 has a function in synaptic plasticity and cognition. A polymorphism in the XBP1 promoter reduces total levels of XBP1 (REF. 151) and was initially linked to psychiatric disorders in Japan^{158,159}, but has more recently been proposed as a risk factor for AD in China¹⁶⁰. This hypothesis is supported by evidence from a mouse model with a conditional deletion of XBP1, which bypasses the lethality of complete XBP1 deletion¹¹⁴. Phenotypic screening of this mouse model identified selective defects in processes related to learning and memory that were associated with altered synaptic transmission in the hippocampus¹⁶¹.

Studies using XBP1s transgenic mice or local delivery of AAV–XBP1s into the hippocampus, showed that the gain of function improved the basal learning and memory capacity of mice. Gene expression analysis of the hippocampus in XBP1-deficient animals indicated no changes in the expression of typical UPR target genes, but several factors involved in neuronal plasticity were dramatically downregulated¹⁶¹. Brain-derived growth factor (BDNF) was among these downregulated factors, and evidence showed that it is directly regulated by XBP1s, suggesting that BDNF mediates this novel activity of the pathway on synaptic plasticity. These observations indicate that XBP1 is part of a molecular network that influences normal synaptic plasticity and memory functions. This concept has been applied to a model of AD, in which expression of XBP1s reversed memory deficits and increased dendritic spine density and synaptic transmission in the hippocampus¹⁶². Furthermore, evidence in cell culture systems suggests that XBP1 mRNA splicing operates downstream of BDNF signalling¹⁶³, regulating the expression of neuropeptides and synaptic genes¹⁶⁴.

Neuroinflammation and axonal damage

All PMDs are characterized by increased neuroinflammation associated with the activation of astrocytes and microglia, and with the infiltration of immune cells into

the brain^{3,4}. In addition, axonal degeneration is an early pathological event in most PMDs, and occurs before neuronal loss is evident. However, emerging evidence indicates similar roles for ER stress and the UPR in neuroinflammatory disease and neurological injury. Studies suggest that a similar mechanism of neurodegeneration occurs in multiple sclerosis, and that the UPR contributes to the prevention of axonal damage in models of injury and to preservation of oligodendrocyte function under conditions of chronic ER stress in the brain (Supplementary information S1 (table)).

Several studies indicate that inflammatory damage to the nervous system triggers neurodegeneration as a result of chronic ER stress. In experimental autoimmune encephalitis, genetic manipulation of PERK expression or use of eIF2 α phosphatase inhibitors has demonstrated that activity of this pathway improves oligodendrocyte function and survival^{165–168}.

Mechanical damage to the CNS has also been associated with rapid induction of abnormal ER stress levels, followed by motor dysfunction¹⁶⁹. In the context of experimental spinal cord injury in mice, XBP1 or ATF4 deficiency aggravated motor dysfunction¹⁷⁰. Remarkably, local delivery of AAVs that encode XBP1s into the injured area attenuated motor deficits and was associated with improved oligodendrocyte survival¹⁷⁰. Similarly, expression of ATF4 had positive effects on motor recovery after spinal cord injury, and this effect was associated with reduced oligodendrocyte loss¹⁷⁰. In contusion models of spinal injury in mice, genetic ablation of CHOP or treatment with salubrinal improved oligodendrocyte survival and hindlimb locomotion^{171,172}. However, no protection was observed when the stress-inducible eIF2 α phosphatase was inhibited with guanabenz or via the deletion of GADD34 (REF. 173). In the context of optic nerve crush, PERK deficiency or treatment of mice with ISRIB reduced axonal degeneration¹⁷⁴. Finally, ATF6 α deficiency modulated ER stress levels after spinal cord injury in mice, but had no effect on motor recovery¹⁷⁵. Consistent with these observations, administration of chemical chaperones attenuates ER stress levels in animal models of brain damage and spinal cord injury^{176,177}.

Studies of the PNS have indicated that the UPR improves axonal regeneration. In a model of sciatic nerve crush, XBP1s expression accelerated axonal regeneration¹⁷⁸, and the effect was associated with remyelination and removal of axonal debris, possibly as a result of increased macrophage infiltration and augmented levels of the MCP-1 chemokine. In agreement with this concept, improvement of ER proteostasis by the overexpression of ERp57 delayed peripheral nerve degeneration in mice¹⁴⁰. Unexpectedly, ATF4 deficiency did not influence axonal degeneration and regeneration after sciatic nerve crush¹⁷⁸.

Together, these studies of inflammatory disease and neuronal injury suggest that chronic ER stress represents a general mechanism of neurodegeneration that is triggered not only by the accumulation of disease-related misfolded proteins but also by the proinflammatory environment observed in neurodegenerative disease. Oligodendrocytes are the main cell type that undergoes ER stress-related damage as a result of these

inflammatory conditions and mechanical injury that is linked to pathological conditions, consistent with the fact that this cell type has high secretory activity and produces large amounts of lipids and myelin proteins.

Perspective and conclusions

The first functional studies to link ER stress with brain damage were published over 18 years ago^{29,179}. For almost a decade, ER stress was viewed as a pro-degenerative event that occurred downstream of the primary aetiology, but the availability of genetically modified mice and small molecules to selectively target specific UPR mediators have revolutionized the field, revealing a complex scenario in which the UPR can have distinct outcomes in different diseases and when different signalling components are manipulated. Moreover, the UPR can have opposite effects depending on the disease stage, first operating as a pro-survival factor to sustain proteostasis, but shifting towards the promotion of cell damage during late symptomatic phases. Importantly, ER stress is not only a consequence of deleterious effects on the secretory pathway, but also can trigger a vicious cycle by increasing aggregation of disease-associated proteins. In this context, activation of the UPR can counterbalance protein aggregation by improving protein folding and promoting clearance pathways, such as ERAD and autophagy. As most of the proteins linked with PMDs spread through the brain by a prion-like mechanism¹⁸⁰, the possible role of ER stress in the process of disease propagation through protein misfolding remains to be determined.

Studies indicate that alterations in the function of the ER contribute to the aetiology of several neurodegenerative diseases. The discovery of mutations in genes that encode ER chaperones in ALS and components of the UPR in AD indicates that the mechanisms for surveying and sustaining ER proteostasis are fundamental to the maintenance of function in specific neuronal populations. Remarkably, mutations in *ATF6* were discovered as a cause of achromatopsia, an autosomal recessive disorder associated with colour blindness, photophobia and severely reduced visual acuity; this observation indicates that mutations in central components of the UPR can initiate disease^{181–183}. This role becomes highly relevant in light of the new evidence that the UPR modulates synaptic plasticity and neuronal connectivity, and the possible relevance to autism spectrum disorders (ASDs). Studies have also indicated the involvement of UPR signalling in brain development, with effects on neuronal

differentiation, neurogenesis and dendrite outgrowth (reviewed in REF. 184), which might have implications for neurodevelopmental diseases. Of note, genetic studies of ASDs have indicated that synaptic dysfunction is one of the molecular pathways underlying this neurodevelopmental disorder. Polymorphisms in neuroligins (NLGNs) have been associated with an increased susceptibility to ASD, and a point mutation in *NLGN3* leads to partial retention of the mutant protein in the ER, which induces UPR signalling^{185,186}.

Several therapeutic approaches to reducing ER stress are under development. A variety of small molecules are available to stimulate or inhibit PERK signalling, with outstanding results in various preclinical models of neurodegeneration. Small molecules to inhibit IRE1 α have also been identified, but have not yet been tested in brain diseases. Targeting the UPR presents challenges, as the pathway has a role in the physiology of various cell types and organs, such as the liver and pancreas, so serious adverse effects are predicted with long-term administration of drugs that target the UPR. Gene therapy is emerging as an attractive alternative to small molecules that could bypass their pleiotropic effects because it enables selective targeting of specific brain regions. Outstanding results have been reported from the use of AAVs to deliver active XBPs or BiP in several models of neurodegenerative disease, with no adverse effects reported to date. The field of AAV-based gene therapy has undergone enormous expansion since 2015, and several clinical trials are in development in PD and spinal muscular atrophy; the approaches in these trials involve intracerebral injection of AAVs, and no adverse effects have been reported. Furthermore, the expected FDA approval of the first gene therapy for Leber disease will accelerate development in all regulatory aspects of AAV-based therapies.

The long-term consequences of administering the current UPR-targeting drugs and biologics will need to be determined before the field moves forward to using these agents in the clinic. Particular attention should be given to the consequences of manipulating the UPR for basal motor and cognitive functions of the nervous system. In addition, the UPR has a role in sustaining the growth of glioma tumours¹⁸⁷, so stimulation of the UPR could increase the risk of glioma, and this aspect should be evaluated in long-term studies. Future efforts are needed to systematically define the components of the ER proteostasis network that should be targeted for optimal disease intervention.

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