

## REVIEW ARTICLE

# The unfolded protein response in neurodegenerative disorders – therapeutic modulation of the PERK pathway

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**Keywords**

ER stress; neurodegeneration; neuroprotection; PKR-like endoplasmic reticulum kinase; unfolded protein response

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(Received 20 December 2017, revised 25 January 2018, accepted 20 February 2018)

doi:10.1111/febs.14422

The unfolded protein response (UPR) is a highly conserved protein quality control mechanism, activated in response to Endoplasmic Reticulum (ER) stress. Signalling is mediated through three branches, PERK, IRE1, and ATF6, respectively, that together provide a coordinated response that contributes to overcoming disrupted proteostasis. PERK branch activation predominantly causes a rapid reduction in global rates of translation, while the IRE1 and ATF6 branch signalling induce a transcriptional response resulting in expression of chaperones and components of the protein degradation machinery. Protein misfolding neurodegenerative diseases show disruption of proteostasis as a biochemical feature. In the brains of animal models of disease and in human post mortem tissue from many of these disorders, markers of UPR induction, particularly, the PERK pathway can be observed in close association with disease progression. Recent research has revealed dysregulated UPR signalling to be a major pathogenic mechanism in neurodegeneration, and that genetic and pharmacological modulation of the PERK pathway results in potent neuroprotection. Targeting aberrant UPR signalling is the focus of new therapeutic strategies, which importantly could be beneficial across the broad spectrum of neurodegenerative diseases.

## Introduction

Neurodegenerative diseases vary in their clinical, pathological and biochemical signatures. However, a common feature of the protein misfolding neurodegenerative disorders is the misfolding and aggregation of disease-specific proteins, associated with the death of neurons. The disease-specific misfolded protein/s in the

brain include amyloid-beta ( $A\beta$ ) and hyperphosphorylated tau in Alzheimer's disease (AD),  $\alpha$ -synuclein in Parkinson's disease (PD), TDP-43, FUS or SOD1 in amyotrophic lateral sclerosis (ALS) and the prion protein (PrP) in prion diseases. How these misfolded and aggregated protein/s lead to extensive neuronal loss

**Abbreviations**

$A\beta$ , Amyloid-beta; AD, Alzheimer's disease; ALS, Amyotrophic Lateral Sclerosis; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; BACE1, beta-secretase 1; BiP, binding immunoglobulin protein; CHOP, CCAAT-enhancer-binding protein homologous protein; eIF2, eukaryotic initiation factor 2; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; ERSE, ER stress element; GADD34, Growth arrest and DNA damage-inducible protein 34 kDa; GEF, guanine nucleotide exchange factor; GLS, golgi localization sequence; HD, Huntington's disease; LTP, long-term potentiation; ISRIB, integrated stress response inhibitor; IRE1, inositol requiring enzyme 1; PD, Parkinson's disease; PERK, protein kinase RNA (PKR)-like ER kinase; PrP, prion protein; PSP, progressive supranuclear palsy; RIDD, regulated IRE1-mediated decay; TDP43, Tar DNA binding protein 43 kDa; UPR, unfolded protein response; XBP1, X-box binding protein 1; XBP1s, XBP1 spliced.

has been relatively unclear until recently, hence, disease-modifying therapeutics remain frustratingly elusive. An extensive body of research and many high-profile drug trials have directly targeted these disease-specific misfolded proteins, and while this approach is supported by numerous preclinical studies, clinical efficacy appears to be lacking. A $\beta$  plaques in particular has been targeted in many trials for AD with little clinical success [1–3], this is despite the relative efficacy of the A $\beta$  reduction, forcing a re-examination of the causes of neurodegenerative disease and whether other pathogenic mechanisms may better explain the neurotoxicity observed.

As well as the numerous disease-specific features, these disorders share many pathological similarities, such as microglial activation, neuroinflammation and disruption to proteostasis. An approach that targets a common mechanism of neurodegeneration would be beneficial in each specific disease that aberrant physiological process is observed in, making a multifunctional treatment an extremely attractive prospect. This review will summarize recent evidence for the role of one of these common processes – the dysregulation of the unfolded protein response (UPR) due to protein misfolding – in neurodegenerative disorders. The review will also discuss current and future therapeutic targeting of the UPR, focusing mainly on PERK and its downstream effectors.

## The Endoplasmic Reticulum and the unfolded protein response (UPR)

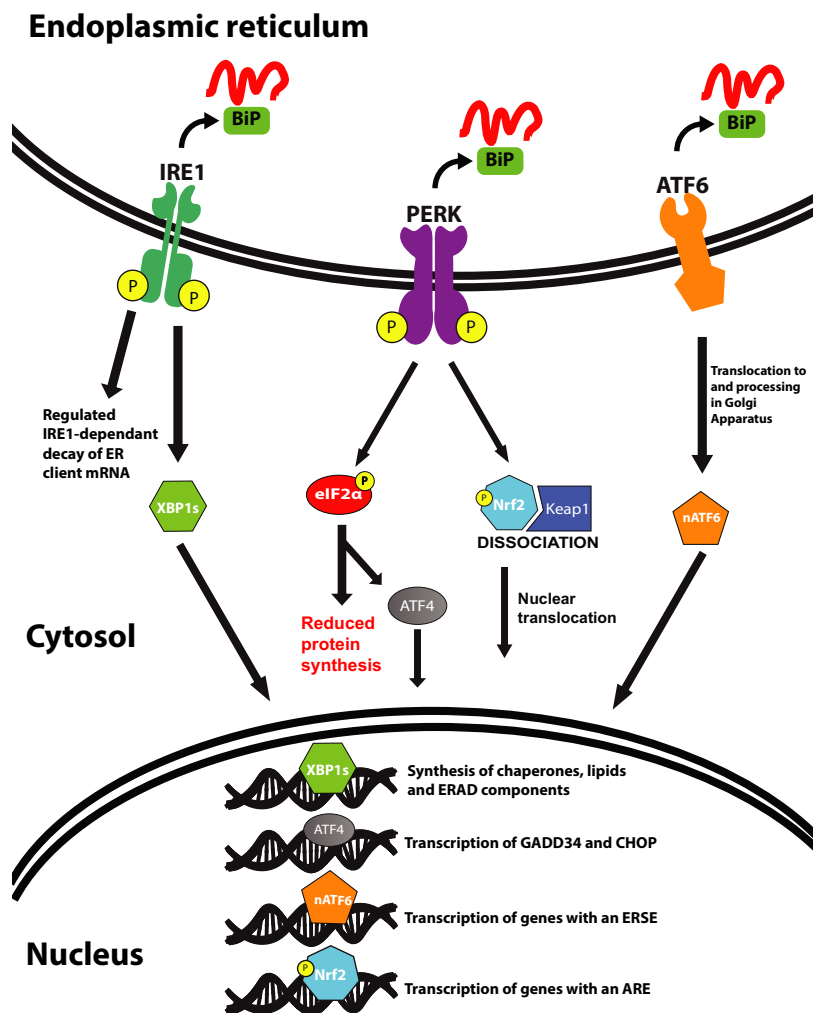
The Endoplasmic Reticulum (ER) is a large dynamic organelle with multiple functions including calcium storage and lipid metabolism. Most importantly, the ER is a major site of cellular protein synthesis. Secreted proteins and integral membrane proteins are synthesised and folded within the ER, as are some cytosolic proteins [4]. Disruptions to the homeostasis of protein folding lead to the accumulation of misfolded proteins in the ER, resulting in the activation of three highly conserved signal transduction pathways, which react to and help cells recover from ER stress, these three signalling pathways are collectively known as the unfolded protein response (UPR) [5–7]. The UPR is the main quality control mechanism in the ER and through this response the cell attempts to maintain or recover proteostasis. However, if this is not possible, for instance, if the ER stress is sustained and misfolded protein cannot be refolded or degraded, the cell will undergo apoptosis [5,7]. Three ER transmembrane proteins transduce signals resulting from ER stress from the ER lumen to the cytosol; inositol requiring enzyme

1 (IRE1), activating transcription factor 6 (ATF6) and PKR-like endoplasmic reticulum kinase (PERK). One master regulator, Binding immunoglobulin Protein (BiP), binds to the ER luminal face of all three UPR transducers under “normal” unstressed conditions. This binding is believed to maintain PERK and IRE1 in their monomeric inactive conformation and masks a golgi localization signal (GLS) on ATF6 [8]. The presence of misfolded protein/s in the ER facilitates BiP dissociation from the three UPR effectors (Fig. 1), allowing BiP to bind misfolded proteins directly. The effects of this are twofold; firstly, BiP maintains the misfolded polypeptide in a folding-competent state for refolding by other chaperones such as GRP94 or for ejection from the ER for degradation [8]. Secondly, BiP dissociation causes activation of IRE1, ATF6 and PERK and their associated downstream pathways. There is also evidence that unfolded proteins can bind the ER luminal regions of both PERK and IRE1 directly [6,9], this is believed to play a role in their activation and even protein folding [10].

## IRE1

IRE1 is the most conserved ER stress sensor of the UPR; it contains two enzymatic domains; a kinase domain and an endonuclease domain, both located in its cytoplasmic region [11–13]. Dissociation of BiP from IRE1 leads to dimerization of IRE1 and *trans*-phosphorylation by its kinase domain, which in turn activates its endonuclease domain. The most studied function of the endonuclease domain is cleavage of 26 base pairs from X-box binding protein 1 (XBP1) mRNA (Fig. 1). This results in a frame shift in XBP1 mRNA leading to translation of a potent bZIP transcription factor, XBP1s [12–14]. XBP1s binds endoplasmic reticulum stress elements (ERSE) in DNA and promotes the induction of endoplasmic reticulum-associated degradation (ERAD) components needed for the degradation of misfolded protein by the 26S proteasome. Molecular chaperones such as BiP and GRP94 are also under the control of ERSEs. XBP1s also indirectly regulates biogenesis and growth of the ER by increasing the activity of phospholipid synthesizing enzymes [11], increasing the folding capacity of the ER in times of stress.

Another emerging cellular function of IRE1 is control of regulated IRE1-dependent decay of mRNA (RIDD) (Fig. 1). RIDD involves cleavage of ER-associated mRNAs, however, in contrast to XBP1, the mRNAs are degraded [14,15]. RIDD is thought to decrease the abundance of incoming ER proteins hence further reducing folding load in the ER [16].



**Fig. 1.** The unfolded protein response. The presence of misfolded proteins in the ER causes the dissociation of BiP from the three unfolded protein response (UPR) sensors, protein kinase RNA (PKR)-like ER kinase (PERK), IRE1 and activating transcription factor 6 (ATF6), leading to their activation. IRE1 activation results in splicing of X-box binding protein 1 (XBP1), spliced XBP1 (XBP1s) activates many UPR target genes related to protein folding, lipid synthesis, protein translocation into the ER and ER-associated decay (ERAD). PERK phosphorylates eIF2 $\alpha$ , resulting in translational repression and the upregulation of a subset of UPR target genes including activating transcription factor 4 (ATF4) and the proapoptotic CHOP. ATF6 activation targets genes under the control of an endoplasmic stress response element (ERSE), including molecular chaperones (such as BiP) and components of the ERAD system.

## ATF6

ATF6 is found in an inactive form in the ER membrane in complex with BiP. BiP binds to and blocks two golgi localization sequences (GLS) found on the luminal face of ATF6, preventing trafficking of ATF6 to the golgi leading to its retention in ER membrane. Upon the dissociation of BiP, ATF6 is trafficked to the golgi apparatus where it undergoes processing to its active form. It is cleaved twice, first by luminal serine protease site one protease (S1P), then by intramembrane metalloprotease site 2 protease (S2P)

[6,8,17]. The cleaved cytosolic fragment (nATF6) contains a bZIP domain and translocates to the nucleus to act as a transcription factor. Here, it binds the ATF/cAMP response element and ERSE [8], upregulating several chaperone proteins and prosurvival targets such as Herp, BiP and P58<sup>IPK</sup> [8].

## PERK

In a similar manner to IRE1, dissociation of BiP from PERK's ER luminal face leads to dimerization, inter-dimer *trans*-phosphorylation and activation of its

cytosolic kinase domain [9,18]. Recent evidence also suggests that the PERK protein can form a tetramer which further increases its kinase activity [19]. In the context of the UPR, PERK's main cellular target is eukaryotic Initiation Factor 2 $\alpha$  (eIF2 $\alpha$ ) (Fig. 1). PERK phosphorylates eIF2 $\alpha$  at serine-51, resulting in a temporary reduction in global protein synthesis allowing the cell time to refold or degrade any unfolded protein in the ER. A small subset of genes is upregulated in response to phosphorylation of eIF2 $\alpha$  (Fig. 1), this is reliant on regulatory features found in their 5' untranslated regions (5' UTR).

### Translational control by phosphorylation of eIF2 $\alpha$

eIF2 $\alpha$  forms the heterotrimeric eukaryotic initiation factor 2 (eIF2) when complexed with eIF2 $\beta$  and eIF2 $\gamma$ . eIF2 is an essential constituent of ternary complex, which is required for the initiation of translation of an mRNA into a polypeptide [20]. eIF2 is bound by either GDP or GTP. In its GTP bound state (eIF2-GTP) eIF2 is able to bind to initiator methionine tRNA (tRNA<sup>i</sup>-Met) to form the complete ternary complex (eIF2-GTP-tRNA<sup>i</sup>-Met) [20–22]. Only when ternary complex is fully formed can it associate with the 40S ribosome to form the preinitiation complex (PIC), without PIC assembly mRNAs cannot be translated.

The widely accepted rate-limiting factor of translational initiation and hence translation itself is the conversion of eIF2-GDP into eIF2-GTP, which is under control of the guanine nucleotide exchange factor (GEF) eIF2B [23]. Phosphorylation of eIF2 $\alpha$  at serine 51 (eIF2 $\alpha$ -P) greatly increases the affinity of eIF2 to eIF2B and this tight association potently inhibits eIF2B's GEF activity [20,23]. This causes an immediate and severe reduction in eIF2-GTP and hence ternary complex, attenuating general translation. The reduction is so severe due to the uneven stoichiometry between eIF2 and eIF2B [24]; eIF2 outnumbers eIF2B in the cytosol meaning a small increase in eIF2 $\alpha$ -P can sequester most, if not all, available eIF2B. Phosphorylation of eIF2 $\alpha$  is a converging mechanism for reducing translation in response to a variety of stressors, it is also phosphorylated by four eIF2 $\alpha$  kinases, signalling through which is collectively termed the integrated stress response [25,26]. These kinases are PERK, protein kinase R (PKR) which responds to viral infection, general control nonderepressible 2 (GCN2) which is activated by UV damage or amino acid deprivation, and heme requiring eIF2 $\alpha$  kinase, which responds to iron deficiency.

Downstream of eIF2 $\alpha$ -P signalling a select group of proteins are upregulated, these include activating transcription factor 4 (ATF4), proapoptotic C/EBP Homologous protein (CHOP) and growth arrest and DNA damage-inducible protein 34 kDa (GADD34). Upregulation of ATF4 is the best studied of this subset of proteins; it is able to escape the translational block due to features found in its 5' UTR known as upstream open reading frames (uORFs) [27]. In response to eIF2 $\alpha$  phosphorylation, ATF4 upregulates a number of key genes including GADD34 and CHOP. GADD34 is a regulatory subunit of protein phosphatase 1 and targets it to dephosphorylate eIF2 $\alpha$ -P as part of a negative feedback loop, the function of which is to restore protein synthesis post ER stress. CHOP is downstream of ATF4 and is itself a transcription factor. It is produced in high quantity after prolonged ER stress, promoting apoptosis as well as ER stress-induced cytokine production in macrophages [28].

### The UPR and neurodegeneration

The presence of unfolded protein in brains of patients suffering with neurodegenerative diseases has been increasingly documented over the last decade [29,30], so it is perhaps unsurprising that recent research has brought the UPR into focus as a converging pathological pathway and therapeutic target across the spectrum of these disorders.

### UPR activation in human neurodegenerative disease

Recently, histological evidence of UPR activation has been reported in post mortem brain tissues from patients with various different neurodegenerative diseases, particularly, the detection of PERK-P and eIF2 $\alpha$ -P [31–34]. Upregulation of PERK branch UPR markers in these disorders is associated temporally and spatially with the accumulation of misfolded and aggregated protein [33,35], the authors observed high levels of P-PERK and eIF2 $\alpha$ -P immunoreactivity in the pons of progressive supranuclear palsy (PSP) patients and in the hippocampus of Alzheimer's sufferers in comparison to nondiseased control brains. A genome-wide association study searching for common variants influencing the risk of PSP identified a single-nucleotide polymorphism in intron 2 of the PERK gene *EIF2AK3* [36]. Patient-derived lymphoblastoid cell lines with this mutation showed a stronger UPR stress response than controls, suggesting that this

variant may increase the risk of tauopathy via overactivation of the PERK pathway of the UPR.

Other studies have shown UPR activation is an early event in tauopathies, AD and also in PD [31,37–39]. Immunoreactivity of ER stress markers P-PERK, P-eIF2 $\alpha$  and P-IRE1 was observed in pretangle hippocampal neurons in several human cases of AD, AD with Lewy body pathology and vascular dementia, and this immunoreactivity was most abundant in neurons with that contained ptau, the authors suggest that chronic UPR activation is involved in both tau phosphorylation and AD pathogenesis from early disease points. P-IRE1 correlates with AD disease pathology and IRE1 also exerts control on cellular production of Amyloid Precursor Protein (APP), cleavage of which results in beta-amyloid [40]. P-PERK and eIF2 $\alpha$ -P immunoreactivity have been observed in dopaminergic neurons of the substantia nigra in brain tissue of PD patients that was not present in control brains [32]. P-PERK was colocalized with  $\alpha$ -Synuclein in disease samples, which is often misfolded in disease-forming aggregates. In addition to this, [34] found high levels of UPR markers such as BiP and CHOP in motor neurons taken from the spinal cords of sporadic ALS patients. Taken together these studies suggest that UPR activation could be a pathological mechanism across the whole spectrum of neurodegenerative diseases, occurring independently of the specific disease-causing misfolded protein (Table 1).

Pathological observations of upregulation of the UPR in human disease are very interesting, however, it is difficult to establish a causal relationship from these data alone. Hence, the above findings have been used to inform studies in animals to attempt to further elucidate the role of the UPR and its signalling components in neurodegeneration.

### UPR activation in animal models of neurodegenerative disease

Numerous models of neurodegeneration have been used to understand the pathogenic role of UPR signalling, with a wealth of data revealed from mouse studies, assisted by experiments in *Drosophila* and other rodent models. ER stress is a common feature in animal models of neurodegeneration, recapitulating the observations from human post mortem studies. Raised levels of PERK-P and its downstream effectors are observed in prion-infected mice [41,42] in rTg4510 mice, which overexpress human tau mutation harbouring the P301L mutation associated with frontotemporal dementia [43,44], in 5xFAD mice that express five AD-linked mutations [45] as well as in mutant

SOD1-expressing mice [46]. Thus, both human post mortem tissue and mouse models of neurodegenerative diseases show a correlation between UPR activation, particularly induction of the PERK branch, and neuropathology.

Insight into the mechanistic relevance of these observations comes from recent work in prion-diseased and frontotemporal dementia mice in particular. Studies testing molecules targeting various parts of the PERK pathway have shown promise in numerous disease models.

### Prion disease

Many mouse models of disease employ overexpression of a disease-specific protein or mutant, which results in variable phenotypic modelling of disease as well as limited clinicopathological relevance (reviewed in refs. [47,48]). Prion disease in mice, in contrast, recapitulates all aspects of human prion disease from infectivity and biochemistry, to pathology and clinical signs. Mouse prion disease, therefore, provides a model in which the relationship between protein misfolding and neuronal loss in disease can be studied directly. Mice inoculated with prions follow a stereotypical and well-studied disease time course. Tg37<sup>+/-</sup> mice overexpress the mouse wild-type prion protein (PrP) approximately threefold and succumb to prion disease 12 weeks after inoculation with Rocky Mountain Laboratory prion-infected brain homogenate. Prion-inoculated Tg37<sup>+/-</sup> mice display synapse loss at 7 weeks postinoculation (wpi) in CA1 hippocampal neurons. PERK activation and phosphorylation of its major target eIF2 $\alpha$  are observed from 9wpi, preceding neuronal loss from 10wpi [41]. Genetic intervention by lentivirally expressing human GADD34, the eIF2 $\alpha$ -targeting component of protein phosphatase 1, restores translation rates, prevents behavioural deficits and the onset of clinical signs as well as preventing neurodegeneration. Administration of salubrinal (a repressor of eIF2 $\alpha$  dephosphorylation) increases eIF2 $\alpha$ -P, which exacerbates neurodegeneration and reduces survival time in these mice. Importantly, these observations were also replicated in prion-infected mice with wild-type levels of prion protein, demonstrating that this is not a gene dosage effect [41].

### Frontotemporal dementia

The rTg4510 mouse model of frontotemporal dementia (FTD) overexpresses human four-repeat P301L mutant tau in the forebrain [49,50]. Tissue-specific expression of the mutant tau is driven by crossing the tg4510 mice

**Table 1.** UPR markers are observed in human neurodegenerative diseases as well as numerous mouse and fly models of disease.

Species	Disease	Markers	Location	Reference
Human	AD	P-PERK, eIF2 $\alpha$ -P	Hippocampus	[33]
	AD	P-IRE1	Hippocampus CA1	[40]
	AD, AD with lewy body pathology, vascular dementia	P-PERK, eIF2 $\alpha$ -P, P-IRE1	Hippocampus	[31]
	PD	P-PERK, eIF2 $\alpha$ -P	Substantia Nigra	[32]
	PSP	P-PERK, eIF2 $\alpha$ -P	Pons	[33]
	sALS	BiP, CHOP	Spinal cord	[34]
	sALS	CHOP	Spinal cord	[57]
Mouse	AD	P-PERK, eIF2 $\alpha$ -P, ATF4	Hippocampus	[45,55]
	ALS	P-PERK, eIF2 $\alpha$ -P, ATF4, GRP78, CHOP, XBP1s	Spinal cord	[46,56,58]
	Charcot-Marie-Tooth 1B	GRP78, CHOP, XBP1s	Spinal cord	[56]
	FTD	P-PERK, eIF2 $\alpha$ -P, ATF4	Hippocampus, forebrain	[43,44,80]
	Prion	P-PERK, eIF2 $\alpha$ -P, ATF4, CHOP	Hippocampus	[41,42,70,80]
	PD	BiP, GRP94, PDI	Spinal cord, brain stem	[61]
<i>Drosophila</i>	ALS	eIF2 $\alpha$ -P		[59]
	PD	P-PERK, eIF2 $\alpha$ -P, ATF4, CHOP		[89]

with a neuronal-specific promoter (CAMKIIa) tetracycline-controlled transactivator-expressing mouse line, meaning the mutant tau is neuronally expressed with particularly high levels in the forebrain and hippocampus. At around 2.5 months of age spatial memory is impaired, when performance is examined using a Morris water maze. At 4 months of age tangle-like tau inclusions can be seen in the cortex and can be observed in the hippocampus from around 5.5 months of age. This correlates with approximately 60% loss of hippocampal neurons at 5.5 months [49]. Significant motor impairment is present at 6 months followed by cortical cell loss at 8.5 months and gross forebrain atrophy at 10 months of age [50]. PERK-mediated eIF2 $\alpha$  phosphorylation and increased levels of the downstream effector ATF4 is evident at 6 months of age [44]. P-PERK, eIF2 $\alpha$ -P as well as BiP are also significantly increased in hippocampus and forebrain at 9 months of age [43].

### Alzheimer's disease

Genetic reduction in PERK in 5XFAD mice (an AD model that expresses five mutations linked to familial AD) has been shown to rescue memory deficits and cholinergic neurodegeneration [45]. Interestingly PERK haploinsufficiency also prevented elevations in beta-secretase 1 (BACE1), one of the secretases involved in A $\beta$  production. This led to reduced A $\beta$  levels and plaque burden. This is hypothesized to be because BACE1 is upregulated during eIF2 $\alpha$  phosphorylation in a similar manner to ATF4 [45]. Aberrant phosphorylation of eIF2 $\alpha$  has also been implicated in

memory loss, a key early clinical feature in AD as well as many related neurodegenerative diseases. Long-term synaptic plasticity and memory formation rely on *de novo* protein synthesis so will be negatively affected by sustained phosphorylation of eIF2 $\alpha$  [51–53]. Downstream PERK pathway effector ATF4 is a repressor of cAMP response element binding protein-mediated gene expression, which is required for synaptic plasticity as well as learning. In mice, reducing the phosphorylation of eIF2 $\alpha$  via the heterozygous expression of an eIF2 $\alpha$  phospho-null mutant enhances synaptic plasticity, spatial learning and memory by lowering the threshold for long-term potentiation (LTP) [54]. Conversely, blocking the dephosphorylation of eIF2 $\alpha$  impairs both late-LTP and long-term memory in mice [54]. In a similar study, genetic deletion of PERK and another eIF2 $\alpha$  kinase GCN2 improved synaptic plasticity and spatial memory in a mouse model of AD [55].

Further evidence of UPR involvement in AD comes from the APP/PS1 mouse model of AD. Genetic deletion of PERK or GCN2 resulted in reduced eIF2 $\alpha$ -P which prevented impairments in synaptic plasticity [55]. The reduction of eIF2 $\alpha$ -P also prevented deficits in spatial memory.

### Amyotrophic lateral sclerosis (ALS)

A number of mouse models of ALS suggest a role for the UPR in neuronal cell death, [46] studied three models of SOD1 mutant transgenic mice in which point mutations G93A or G85R were expressed at varying levels. They found that ALS “vulnerable” neurons were selectively prone to ER stress and that this

ER stress is among the earliest observable cellular defects, which begins from a young age. ER stress markers GRP78, XBP1s and CHOP were observed to be elevated in the same G93A SOD1 mutant mice [56]. Levels of CHOP have been observed to be considerably higher than nondisease controls in the spinal cords of sporadic human ALS sufferers and mutant SOD1 mice [57]. The elevated CHOP levels are primarily found in motor neurons and microglia. SOD1 G85R mice that are haploinsufficient for PERK show a significantly accelerated disease onset and reduced survival [58], this was combined with earlier SOD1 aggregation suggesting PERK activation can have a protective effect during ALS disease progression.

eIF2 $\alpha$ -P is upregulated by toxic TDP43 aggregates in *Drosophila* and inhibition of PERK mitigates this toxicity in the *Drosophila* as well as cultured mammalian neurons [59]. Contrasting data show ATF4 can play a protective role during ALS [60]. Although ATF4 deficiency in mutant SOD1 G85R mice reduced birth rate, mice that were born appeared more resistant to ALS and had longer lifespans than mutant mice expressing normal levels of ATF4 [60]. ATF4 knockdown increased aggregation of SOD1 at the end stage of disease.

### Parkinson's disease (PD)

An  $\alpha$ -Synuclein mouse model of PD in which the A53T mutant of human  $\alpha$ -Synuclein is placed under the control of the PrP promoter also shows pathogenic signalling by the ER is involved in neurodegeneration. Disease onset in these mice, including axonal degeneration and neuronal loss, coincided with the induction of ER chaperones [61]. However, this upregulation was not observed alongside increased phosphorylation of eIF2 $\alpha$ . Interestingly, salubrinal, which increases levels of eIF2 $\alpha$  phosphorylation, was protective in this model of  $\alpha$ -synucleinopathy, administration of salubrinal delayed disease onset and reduced accumulation of  $\alpha$ -synuclein in the ER [62]. A study in a rat PD model in which ATF4 was introduced to the dopamine neurons in the substantia nigra via an adenovirus showed sustained expression of ATF4 resulted in severe nigrostriatal degeneration [63]. ATF6 processing has also been implicated in Parkinson's pathology; cellular PD models show  $\alpha$ -synuclein inhibits processing of ATF6 and this results in an impaired ERAD function and increased proapoptotic signalling [64]. This suggests that some facets of UPR signalling could be protective.

ER stress is also implicated in *Drosophila pink1* and *parkin* mutant models of PD, [65] found that defective

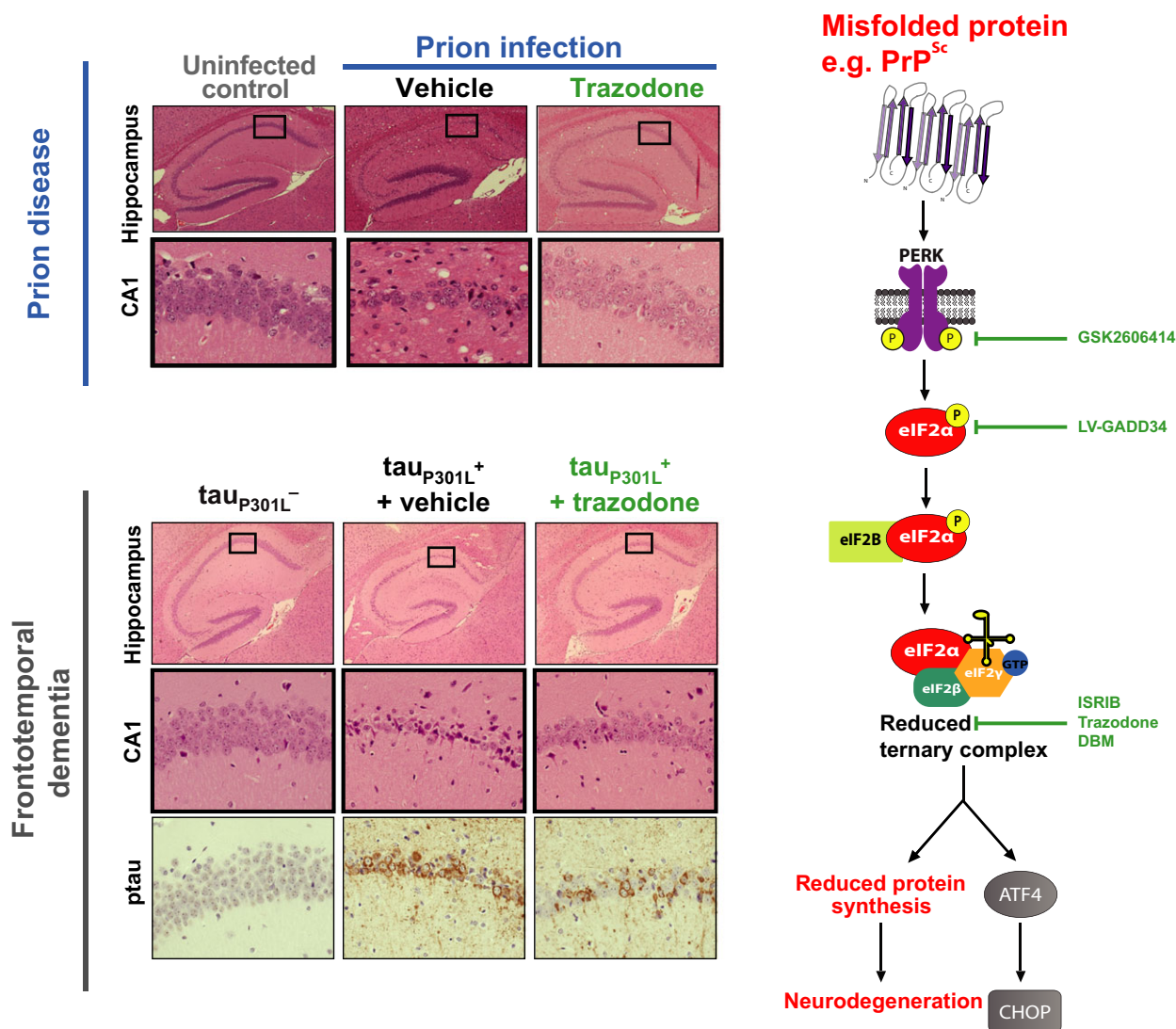
mitochondria fuse with the ER via mitofusin bridges. These bridges promote activation of the UPR and the authors found reducing mitofusin contacts was neuroprotective, they also show that inhibition of PERK using small molecule GSK2606414 was also neuroprotective independent of the mitofusin bridges.

### Huntington's disease

ER stress is an early event in presymptomatic HD mouse models and is evident in post mortem brains of HD patients [66]. BiP and CHOP are both observed as being upregulated in HD patient's brains in comparison with control brains [66]. Expanded polyglutamine expansions, such as those found in disease-causing huntingtin (htt), are thought to result in ER stress by interfering and inhibiting ERAD components [67,68]. It has been shown recently that it is the soluble form of htt that activates ER stress, this occurs before formation of insoluble aggregates. Suggesting it is the formation of small oligomers is that is cytotoxic and that larger aggregates could even be protective [69].

### Therapeutic modulation of the PERK pathway

As discussed above, genetic modulation of the PERK-eIF2 $\alpha$  pathway can prevent neurodegeneration in prion-infected mice. In one study prion-infected Tg37<sup>+/-</sup> mice were treated with GSK2606414, a potent and bioavailable PERK inhibitor [70,71] (Fig. 2). This compound effectively reduced levels of P-PERK and eIF2 $\alpha$ -P, resulting in protection from hippocampal neuronal loss and preventing the emergence of clinical signs of prion disease, even when dosed after the emergence of early neurological disease indicators. Deficits in burrowing, which is a hippocampus-dependent measure of motivation, were reversed by GSK2606414 as was spatial memory loss as measured by novel object recognition. This is particularly exciting as both human and mouse prion disease are particularly devastating when compared with other neurodegenerative diseases, often only taking months from diagnosis to death in humans [72], demonstrating the efficacy of this approach. However, the drug was particularly toxic to the pancreases of the mice likely due to on-target effects of the compound. The pancreas is reliant on a robust ER stress response for regulation of the high levels of protein synthesis and output, inhibition of which resulted in weight loss and mild hyperglycaemia making systemic PERK inhibition unsuitable for therapeutic application.



**Fig. 2.** Trazodone is neuroprotective in prion-infected and frontotemporal dementia mice, haematoxylin and eosin staining shows reduced neuronal loss in hippocampus CA1 as well as reduced ptau in neuronal ribbon. Modulation of the PERK-eIF2 $\alpha$  pathway prevents neurodegeneration. Disease-associated misfolded proteins activate protein kinase RNA (PKR)-like ER kinase (PERK) signalling in many neurodegenerative diseases. Chronic translational repression is associated with neurodegeneration in several experimental models and human post mortem tissue samples. Overexpression of GADD34 prevents neurodegeneration in prion-infected Tg37<sup>+/-</sup> mice by reducing eIF2 $\alpha$ -P. PERK inhibitor GSK2606414 prevents neurodegeneration in prion-infected Tg37<sup>+/-</sup> mice, rTg4510 frontotemporal dementia mice as well as drosophila models of ALS and PD. Compounds promoting ternary complex formation (ISRIB, Dibenzoylmethane and Trazodone) restore translation and are neuroprotective.

PERK inhibition using GSK2606414 is neuroprotective in two PD *drosophila* models, the compound prevented neuronal death in pink1 and parkin mutant flies [65]. TDP43 toxicity is ameliorated by GSK2606414 in mutant flies as well as mammalian neurons [59].

GSK2606414 was also profoundly neuroprotective in the rTg4510 mouse model of frontotemporal dementia [44]. Interestingly, the PERK inhibitor also seemed

to reduce the levels of disease-specific protein ptau, possibly via a divergent mechanism of protection. This is thought to be due to an interaction between activation of eIF2 $\alpha$  kinases PERK and PKR and the activation of Glycogen Synthase Kinase 3 $\beta$  (GSK3 $\beta$ ) [73,74] which is a tau kinase linked to phosphorylation at disease relevant epitopes. PERK inhibition also inhibits activation of GSK3 $\beta$  resulting in less pathogenic phosphorylation of tau [44]. Although the PERK inhibitor



is not suitable as a therapeutic because of the on-target effects of pancreatic toxicity, this study acts as a proof of principle that targeting of the PERK pathway may hold promise in a wider variety of neurodegenerative diseases. With a possibility of two mechanisms of action, targeting both cause and effect of neurodegeneration, further study into safer PERK inhibition strategies should be pursued.

A second compound, ISRIB (integrated stress response inhibitor), which acts downstream of eIF2 $\alpha$  phosphorylation [75,76], has been used to successfully delay neurodegeneration in prion-infected mice [42]. ISRIB binds to eIF2B stabilizing it in its dimeric form [77] allowing it to act as a GEF even in the presence of eIF2 $\alpha$ -P. This results in a partial restoration of protein synthesis in ER-stressed conditions, ISRIB also boosts memory in nonstressed conditions [77] as well as reducing eIF2 $\alpha$ -P-dependent stress granule assembly [78]. Administration of ISRIB to prion-infected mice resulted in an increase in survival and marked neuroprotection of hippocampal neurons, reflected in an improvement in various behavioural tests [42]. Importantly, the pancreases of the mice treated with ISRIB were histologically indistinguishable from vehicle-treated mice, suggesting that partial inhibition of the PERK pathway and hence partial restoration of protein synthesis was enough to prevent neurodegeneration without pancreatic toxicity. As ISRIB enhances spatial and fear-associated learning in mice (Sidrauski *et al.* [76]), it seems likely that preventing eIF2 $\alpha$ -P-mediated translational repression therapeutically will also protect against early synaptic failure and memory loss in neurodegenerative disorders, as well as later neuronal cell death. Interestingly, ISRIB has also been successfully used in mouse models of traumatic brain injury, ISRIB reversed hippocampal-dependent cognitive deficits in focal contusion and diffuse concussion models [79]. Unfortunately, the insolubility of ISRIB makes it unsuitable for translation into human patients. This work, however, showed targeting the PERK-eIF2 $\alpha$  pathway partially was enough for neuroprotection with minimal on-target side effects in a plethora of conditions.

A further study aimed at repurposing drugs for use in neurodegeneration identified two molecules. Both partially restore protein synthesis rates during ER stress [80]. Trazodone is a licensed antidepressant and Dibenzoylmethane (DBM) is a curcumin analogue which has been researched as having anticancer properties [81,82]. These compounds were effective in preventing neurodegeneration in both prion-infected and rTg4510 mice. Both Trazodone and DBM were neuroprotective at clinically relevant doses [80] (Fig. 2).

Both compounds partially restored rates of translation and Trazodone also decreased pathogenic ptau in rTg4510 mice similarly to the PERK inhibitor (Fig. 2). The compounds improved performance in a test of spatial memory as well as a burrowing assay alongside increasing survival. The mechanism for this remains unclear but both compounds are thought to act downstream of eIF2 $\alpha$  phosphorylation in a similar albeit not identical way to ISRIB.

However, studies on the therapeutic modulation of proteostasis, specifically the PERK pathway, have yielded interesting results in different mouse models of disease. Sephin 1, which prolongs translational repression, was shown to delay neurodegeneration in an ALS model as well as a model of periphery neuropathy Charcot-Marie-Tooth disease Type 1a [56]. Numerous studies use guanabenz, a similar compound to sephin 1, and show a therapeutic benefit in ALS disease models. Guanabenz extended lifespan, delayed onset of disease symptoms and improved motor performance in separate study using G93A SOD1 ALS mice [83]. A different study using the same mutant mice suggests that guanabenz significantly ameliorates disease and increases survival as well as reducing accumulation of mutant SOD1 [84]. In a contrasting study, guanabenz is protective in SOD1 G93A mutant expressing fibroblasts [85] but accelerates ALS-like disease progression in mice expressing the same mutant SOD1. These studies appear contradictory to the above studies mentioned previously that suggest restoring translation as the key to neuroprotection. One explanation for the difference may be whether the disease-specific protein can escape translational repression by eIF2 $\alpha$ -P, for instance, total levels of PrP are not effected by increasing levels of eIF2 $\alpha$ -P or by modulation of the PERK pathway [41,70]. This means reducing translation as an adaptive mechanism will be ineffectual, as PrP<sup>C</sup> will continue to be synthesized and converted into PrP<sup>Sc</sup>. Total levels of other disease-causing proteins may be reduced by eIF2 $\alpha$ -P meaning less would become aggregated. Another possible explanation may be related to the subcellular compartment that the protein misfolds in. Protein aggregates in different cellular compartments may react differently to modulation of proteostasis. Whether the protein can be refolded/degraded may also be of relevance, proteins that misfold inside the ER may be refolded as a result of the chaperone increase downstream of UPR activation, however, proteins like tau which misfold in the cytosol may not. It is also possible that activation of the UPR before misfolded protein aggregates appear would be beneficial because of accumulation of the various chaperones and components of protein

degradation machinery downstream of ER stress. Further research into this phenomenon is needed to know how a particular disease will react to modulation of proteostasis.

## Discussion

It is clear that disease-modifying treatments for neurodegenerative diseases are desperately needed. Recent failures in clinical trials, particularly those aimed at clearance of disease-specific aggregated protein such as A $\beta$  [1–3] supports the pursuit of additional targets. The wealth of data from various human neurodegenerative disease studies suggests that dysregulation of proteostasis, and more specifically, aberrant signalling by the UPR may be a common pathogenic mechanism in neurodegeneration. However, the pathological significance of this UPR activation in human disease remains largely unknown: whether UPR signalling is a protective mechanism or an active contributor to disease pathogenesis cannot be determined from neuropathological observations alone. In animal disease models modulation of the UPR has shown marked early promise across many models of neurodegeneration *in vitro* and *in vivo*. Targeting the UPR, and in particular reducing activation of the PERK branch, is an exciting prospect. It provides neuroprotection independently of the disease-specific associated protein/s, meaning a single therapeutic could be beneficial in multiple disorders as shown by a growing number of studies [41,42,44,59,65,70,80]. In specific instances, increasing PERK-eIF2 $\alpha$  signalling therapeutically could be beneficial [56]. This apparent contradiction nonetheless reflects the pivotal role of the UPR in neurodegeneration. These studies use different disease models and different proteins, which misfold in separate cellular compartments. The treatment timings across studies can also be influential. These are all factors that may affect study outcome.

The mechanism by which ER stress is activated may also vary across diseases, for instance, misfolded proteins in the ER as well as those that accumulate in the cytosol seem to activate ER stress. Therefore, there may be different mechanisms of ER stress activation and these different mechanisms may respond differently to different therapeutic approaches. One such divergent mechanism of UPR activation is starting to be uncovered. ER-mitochondrial contacts, termed mitochondria-associated ER membranes, are also of increased interest in neurodegenerative diseases. The interactions between the ER and mitochondria have been observed as dysregulated in various disease models (thoroughly reviewed by [86]). Currently, little is

known about the effects of increasing/decreasing these contacts. They may provide a mechanism for the activation of the UPR by mitochondrial dysfunction; a novel observation that would greatly expand the known UPR signalling cascades and provides a new point of potential therapeutic intervention. Mitochondrial dysfunction is also observed in AD [87] and interestingly, genetically or pharmacologically targeting mitochondrial proteostasis increased fitness and lifespan in GMC101 worms (a *Caenorhabditis elegans* model of A $\beta$ <sub>1-42</sub> toxicity [88]) as well as reducing amyloid aggregation in worms, cellular and transgenic mouse models of AD [87].

Partial restoration of protein synthesis by targeting up or downstream of eIF2 $\alpha$ -P seems to be able to prevent neurodegeneration in some but not all protein misfolding neurodegenerative diseases without the pancreatic toxicity associated with systemic PERK inhibition. This is a very exciting concept and a huge step forward in finding thus far elusive treatments for neurodegeneration, to the point where a licensed drug has successfully delayed neurodegeneration in different mouse models. As there are multiple well-validated points of the PERK pathway which could be targeted therapeutically, the next challenge will be identifying how best and at what stage of disease the pathway can be modulated in human diseases.

## Acknowledgements

GRM is funded by the Medical Research Council (MRC 5TR50), by ERC Consolidator award (UPR Neuro) and by the UK DRI.

## Conflicts of interest

The authors declare no conflicts of interest.

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