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REVIEW ARTICLE

# The Integrated Stress Response and Phosphorylated Eukaryotic Initiation Factor 2α in Neurodegeneration

Sarah Bond , BS, Claudia Lopez-Lloreda , BS, Patrick J. Gannon , PhD, Cagla Akay-Espinoza , MD, and Kelly L. Jordan-Sciutto , PhD

**Abstract**

The proposed molecular mechanisms underlying neurodegenerative pathogenesis are varied, precluding the development of effective therapies for these increasingly prevalent disorders. One of the most consistent observations across neurodegenerative diseases is the phosphorylation of eukaryotic initiation factor 2α (eIF2α). eIF2α is a translation initiation factor, involved in cap-dependent protein translation, which when phosphorylated causes global translation attenuation. eIF2α phosphorylation is mediated by 4 kinases, which, together with their downstream signaling cascades, constitute the integrated stress response (ISR). While the ISR is activated by stresses commonly observed in neurodegeneration, such as oxidative stress, endoplasmic reticulum stress, and inflammation, it is a canonically adaptive signaling cascade. However, chronic activation of the ISR can contribute to neurodegenerative phenotypes such as neuronal death, memory impairments, and protein aggregation via apoptotic induction and other maladaptive outcomes downstream of phospho-eIF2α-mediated translation inhibition, including neuroinflammation and altered amyloidogenic processing, plausibly in a feed-forward manner. This review examines evidence that dysregulated eIF2a phosphorylation acts as a driver of neurodegeneration, including a survey of observations of ISR signaling in human disease, inspection of the overlap between ISR signaling and neurodegenerative phenomenon, and assessment of recent encouraging findings ameliorating neurodegeneration using developing pharmacological agents which target the ISR. In doing so, gaps in the field, including cross-talk of the ISR kinases and consideration of ISR signaling in non-neuronal central nervous system cell types, are highlighted.

**Key Words:** Cell fate, eIF2α phosphorylation (p-eIF2α), Integrated stress response (ISR), Neurodegeneration, Neurodegenerative disease pathogenesis, Stress signaling.

## INTRODUCTION

In the current era, the prevalence of neurodegenerative diseases such as Alzheimer disease (AD) and Parkinson disease (PD) is becoming an increasingly heavy burden (1). This is highlighted by the fact that for many disorders the available therapies, if any, are only palliative and not curative or even preventative. This is largely because the proposed molecular mechanisms underlying disease pathogenesis are many, varied, untargetable, or unknown. Many of the known mechanisms converge on a pathway that results in global translation inhibition through phosphorylation of the eukaryotic initiation factor 2α (eIF2α), via competitive inhibition of the guanine exchange factor eukaryotic initiation factor 2B (eIF2B), as part of stress response signaling. In recent years, elevated and/or dysregulated phosphorylation of eIF2α (p-eIF2α) has been extensively associated with many neurodegenerative pathologies (2, 3). Phosphorylation of eIF2α lies downstream of 4 kinases which collectively contribute to the integrated stress response (ISR): protein kinase R (PKR), PKR-like endoplasmic reticulum (ER) kinase (PERK), general control nonderepressible 2 (GCN2), and heme-regulated inhibitor (HRI) (4). Activation of these kinases is aimed to protect against certain cellular stresses, including ER stress, oxidative stress, viral infection, inflammation, and amino acid deprivation (Fig. 1). Intriguingly, many of these stresses are common hallmarks of pathology in numerous neurodegenerative diseases. However, the ISR is traditionally considered an adaptive response to cellular stress, and while downstream signaling is known to include maladaptive and even apoptotic responses that could explain many neurodegenerative phenotypes, there remains debate on whether dysregulated eIF2α phosphorylation is a driver for or a sign of chronic neurodegeneration. Regardless, targeting the pathways associated with eIF2α phosphorylation has yielded encouraging results in attempts to ameliorate neurodegeneration.

From the Department of Biochemistry and Biophysics (SB); Department of Neuroscience (CL-L); Department of Pharmacology (PG), Perelman School of Medicine; Department of Basic and Translational Sciences (CA-E); and Department of Basic and Translational Sciences (KLJ-S), School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Send correspondence to: Kelly L. Jordan-Sciutto, PhD, Department of Pathology, University of Pennsylvania, 240 S. 40th Street, Levy Bldg. Rm 312, Philadelphia, PA 19104; E-mail: jordank@upenn.edu

Sarah Bond and Claudia Lopez-Lloreda contributed equally to this work.

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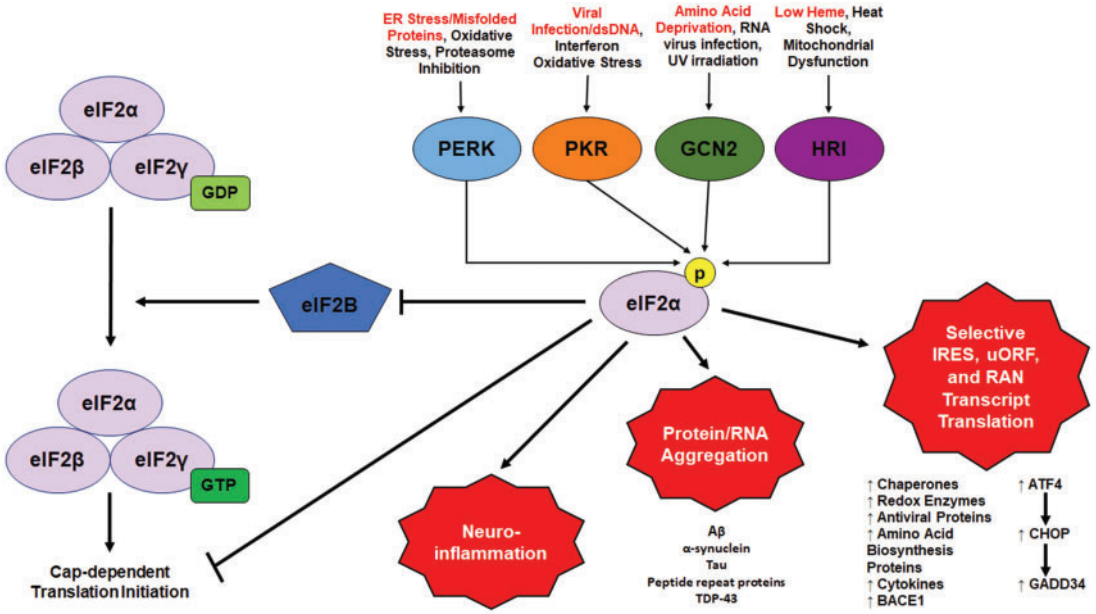


**FIGURE 1.** Canonical integrated stress response signaling. The canonical integrated stress response (ISR) is made up of signaling by 4 stress-activated kinases: protein kinase R (PKR), PKR-like endoplasmic reticulum (ER) kinase (PERK), general control nonderepressible 2 (GCN2), and heme-regulated inhibitor (HRI). Each kinase has a distinct stress which is known to most directly activate it (in red); however, there is extensive overlap between the inductions of each kinase. Also, while each kinase has distinct targets, their main substrate when activated is the alpha subunit of eukaryotic initiation factor 2 (eIF2α). eIF2, when bound to GTP, is a component of the ternary complex required for the initiation of cap-dependent translation. However, p-eIF2α is a competitive inhibitor of eIF2B, the guanine exchange factor responsible for exchanging GDP for GTP on the γ subunit of eIF2 to allow repeat translation initiation. Thus, eIF2α phosphorylation attenuates global translation, while selectively upregulating translation of transcripts with internal ribosome entry sites (IRESs) or upstream open reading frames (uORFs), such as activating transcription factor 4 (ATF4). In turn this induces the upregulation of genes import for resolving stress and, over time with unresolvable stress, inducing apoptosis.

In this review, we summarize the canonical roles of each branch of the ISR and follow up with consideration and evidence of their implications in neurodegenerative pathologies. We also examine the maladaptive outcomes of ISR kinase activation and eIF2α phosphorylation, which might play mechanistic roles in neurodegeneration. Finally, we examine recent findings with emerging pharmacological agents to manipulate these pathways.

## THE ISR-SIGNALING BY 4 DISTINCT STRESS-ACTIVATED KINASES

The ISR is a signaling network of 4 kinases that converges on phosphorylation of eIF2 at serine 51 of the α-subunit, which results in global repression of cap-dependent protein translation (5, 6) (Fig. 1). Composed of 3 subunits, α, β, and γ, eIF2 comprises a ternary complex that binds to the 40S ribosomal subunit to form part of the 43S preinitiation complex when bound to GTP and Met-tRNA (7, 8). After binding to the eIF4F cap recognition complex at the 5′ end of mRNAs, the pre-initiation complex scans the 5′ leader sequence for an AUG start codon. Once an AUG is recognized within a favorable sequence for translation initiation, GTP is hydrolyzed which releases eIF2 from the preinitiation complex and allows the 60S ribosomal subunit to bind the mRNA to initiate the elongation phase of translation (9). Phosphorylation of eIF2α inhibits this process by blocking the dissociation of eIF2 from eIF2B, a guanine nucleotide exchange factor (GEF) that is needed for the exchange of GDP for GTP in the γ subunit of eIF2 (10) and converts eIF2 from a substrate into a competitive inhibitor of eIF2B, thereby slowing the exchange rate of GDP for GTP and limiting the availability of competent ternary complexes to form the preinitiation complex (11).

Importantly, eIF2-mediated repression of protein translation is not universal; a subset of genes is translationally upregulated in response to eIF2α phosphorylation-mediated translational attenuation. These mRNAs escape translational repression through a variety of mechanisms including the presence of internal ribosome entry sites (IRESs) within the 5′ untranslated region (UTR). IRESs are *cis*-activating elements, composed of complex RNA structures, such as hairpins, in the 5′ UTR, which directly recruit the small ribosomal subunit in a cap-independent manner to internal codons with the help of *trans*-acting cellular proteins known as IRES *trans*-acting factors. Another mechanism of this selective upregulation involves small upstream open reading frames (uORFs) of approximately 30 codons in the 5′ UTR of gene transcripts (4, 12). When eIF2α phosphorylation is low and eIF2-GTP levels are high, scanning ribosomes translate the first uORF they encounter, assuming the AUG codon is in an optimal context for initiation. If a transcript contains uORFs upstream of

the authentic start codon, such as that observed with activating transcription factor (ATF) 4, most of the ribosomes will initiate translation at one of the proximal uORFs, which decreases the likelihood that scanning ribosomes will reinitiate in time to begin translation at the authentic AUG start codon; this results in suppression of protein translation. However, when p-eIF2α levels are high and eIF2-GTP is limiting, scanning ribosomes, albeit still initiating translation at the first uORF, are more likely to scan through subsequent uORFs and reinitiate at the authentic start codon due to decreased availability of the preinitiation complex. Thus, decreased availability of the ternary complex/preinitiation complex reduces the translation rate of most cellular mRNAs that contain 1 start codon while simultaneously increasing the translation efficiency of mRNAs with multiple uORFs. This mechanism was first characterized in the yeast homolog of the transcription factor ATF4, GCN4, which contains 4 uORFs, and was subsequently linked to upregulation of a variety of genes involved in stress response, survival, and apoptosis (4, 5, 13–16). Specifically, ATF4 upregulates distinctive genes and transcription factors such as *DDIT3*, the gene for C/EBP homologous protein (CHOP) in an ongoing cascade. Approximately 35%–49% of human gene transcripts are predicted to contain uORFs, indicating that protein translation control via p-eIF2α is likely a widespread mechanism of regulation involved in many cellular functions and an area ripe for investigation of therapeutic interventional targets in human diseases (17).

The first eIF2α kinase identified was HRI, which serves the critical function of balancing the amount of globin production with the available heme levels by inhibiting protein translation in reticulocytes in response to low heme levels (4, 18–20). HRI binds heme at its N-terminus, which triggers the formation of stable HRI dimers (20). When heme levels are low, HRI dimers undergo autophosphorylation, activating the HRI kinase domain and leading to eIF2α phosphorylation and inhibition of global protein synthesis (18). HRI knockout mice display hypersensitivity to heme deficiency but no other physiological abnormalities (21–23).

The second ISR kinase, double-stranded RNA (dsRNA)-dependent PKR, is a cytosolic, constitutively expressed, mammalian kinase that was discovered after the initial observation that extracts from vaccinia virus-infected cells treated with interferon showed enhanced sensitivity to translational inhibition after addition to a cell-free system of exogenous mRNAs or synthetic dsRNA (24–26). Follow-up studies revealed that PKR activation occurred through binding of dsRNA at its N-terminal dsRNA-binding domains, leading to dimerization and autophosphorylation of its kinase domain (25). Downstream p-eIF2α limits translation of viral mRNAs while enhancing the expression of antiviral proteins (4, 27, 28). PKR can also be activated by a variety of dsRNA-independent stressors, including type-1 interferons, oxidative stress, and ER stress, via interaction with the PKR activator PACT (29). PACT is phosphorylated under various stress conditions and utilizes one of its 3 dsRNA-binding domains to interact with and activate PKR (4). Overexpression of PACT sensitizes cells to viral infection and other stresses, whereas knockdown of PACT mitigates stress-induced PKR activation and promotes clonal cell growth. Following activation by PACT, PKR phosphorylates eIF2α and phosphorylates or interacts with a variety of other targets, including signal transducers and activators of transcription, interferon regulatory factor 1, Jun-N terminal protein kinase, ATF3, IkB kinase, and p53, which may mediate some of the observed tumor suppressor activity of PKR (25, 30, 31). Overexpression of PKR leads to potent activation of apoptosis, likely as a protective response against the spread of viral infection. However, despite the ability of PKR to regulate cell survival and death, PKR knockout mice develop normally and display no phenotypic abnormalities (32).

The third ISR kinase, GCN2, was originally identified in yeast as a protein that senses and responds to amino acid deprivation, although it appears to be sensitive to other stressors including viral infection and UV irradiation in mammals (33, 34). GCN2 is activated when uncharged tRNAs bind to its histidyl-tRNA synthetase-related domain and induce dimerization and autophosphorylation to activate the GCN2 kinase domain (4). GCN2-mediated p-eIF2α results in translational upregulation of ATF4, which induces the transcription of numerous genes involved in the amino acid biosynthetic pathway, and protection against oxidative stress (12, 35). GCN2 can sense infection by RNA viruses, such as Sindbis virus and Semliki Forest virus, by binding viral RNA with its histidyl-tRNA synthetase-related domain and inhibits translation via eIF2α phosphorylation (4, 36). One recent study also demonstrated that GCN2 inhibited replication of human immunodeficiency virus (HIV) in vitro and in vivo and was cleaved directly by the HIV-1 protease (37).

The last ISR kinase, PERK, responds to misfolded protein stress in the ER as part of the unfolded protein response (UPR), a homeostatic signaling cascade that responds to ER stress via 3 master regulators: inositol-requiring enzyme (IRE) 1, ATF6, and PERK. These proteins act as sensors and initiate signaling that reestablishes protein homeostasis by increasing the cellular folding capacity (38–40). During UPR activation, following dissociation from binding protein (BiP, also known as GRP78), PERK dimers oligomerize to activate the kinase domain via *trans*-autophosphorylation; this activating phosphorylation results in the subsequent, PERK-mediated p-eIF2α. Repression of global protein synthesis ultimately reduces the production of new mRNAs, with concomitant selective upregulation of protein-coding transcripts containing IRESs or uORFS, such as ATF4; the PERK cascade likewise also leads to the upregulation of folding enzymes, molecular chaperones, and the ER-associated degradation pathway, all aimed to reduce the burden of misfolded proteins and prevent cellular toxicity (4, 41). Importantly, prolonged PERK activation and ATF4 activity are known to induce the proapoptotic transcription factor CHOP. CHOP induces the expression of growth arrest and DNA damage-inducible protein (GADD34), a nonenzymatic cofactor that targets protein phosphatase 1 (PP1) to dephosphorylate eIF2α to provide a negative feedback loop against chronic activation in cases of acute stress (42). PERK plays an important physiological role in secretory cell types, such as pancreatic β cells, which are frequently under a large biosynthetic load in response to varying demands for insulin production and thus have a larger and more developed ER. Missense mutations in *EIF2AK3*, the gene that enco-

des PERK, have been linked to the Wolcott-Rallison syndrome in humans, which is characterized by permanent neonatal diabetes, growth retardation, and pancreatic and skeletal system deficits, accompanied by mental retardation in some cases (43, 44). Similar phenotypic abnormalities were also observed in *EIF2AK3* knockout mice. In addition to alleviating ER stress, PERK plays a critical role in limiting oxidative stress via activation of nuclear factor (erythroid-derived 2)-like 2 (NRF2), a transcription factor that regulates the protein expression of a battery of genes involved in the antioxidant response including detoxifying enzymes such as glutathione S-transferase A2 (GSTA2), heme oxygenase-1 (HO-1), and NADPH quinone oxidoreductase (NQO1) (45). Under normal conditions, NRF2 is kept inactive in the cytoplasm via interaction with and constitutive targeting for degradation by Kelch-like ECH-associated protein 1 (KEAP1) (46). Following phosphorylation by PERK, NRF2 dissociates from KEAP1, migrates to the nucleus, and activates transcription of genes that contain the antioxidant response element in their promoter (47). The significance of the link between ER stress and NRF2 activation is best highlighted by the hypersensitivity of cells lacking NRF2 to compounds that activate ER and oxidative stress (48).

When stress is prolonged and cannot be resolved by the above-mentioned signaling cascades, the ISR enters a second phase of maladaptive signaling and apoptotic induction. ATF4 upregulates the transcription and subsequent expression of the proapoptotic BCL-2 protein NOXA, a plasma membrane proton ATPase, as well as CHOP (3). CHOP induces the expression of the proapoptotic BH3-only BCL-2 family members as well as death receptor 5 (3, 49). Additionally, sustained CHOP expression results in the upregulation of ER oxidoreductase 1α (ERO1α), which further sensitizes cells to death (49–51). ERO1α causes calcium release from the ER by activating the inositol-1,2,5-triphosphate receptor; such calcium dysregulation can induce apoptosis and necrosis (49). Furthermore, translational attenuation downregulates cyclin D1 and BCL-2, thereby sensitizing cells to apoptosis (49). These and other ISR-mediated death pathways, both downstream of and tangential to CHOP, have been reviewed extensively (3, 49, 50, 52, 53).

Altogether, these findings demonstrate that eIF2α phosphorylation by HRI, PKR, GCN2, and PERK is a critical cellular and physiological mechanism of protein translational control that protects cells against a wide variety of stressors but can, however, leads to adverse outcomes when activated in a chronic, uncontrolled fashion. While the precise determinants of the switch from homeostatic to cytotoxic signaling are still being investigated, eIF2α phosphorylation appears to act as a convergence point of many stress inputs, the resultant level of which may dictate cell fate.

## THE ROLE OF ISR KINASES IN NEURODEGENERATION

Elevated and/or dysregulated eIF2α phosphorylation in association with neurodegenerative conditions has been a major focus of research for many years with good reason: Classic neurodegenerative conditions, such as AD, PD, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Huntington disease (HD), and prion disorders, are characterized both clinically and in postmortem patient pathology by chronic neuroinflammation, disturbances in proteostasis, and oxidative stress (41, 54–59), which have been shown to be ISR activators as well. While ISR activation converges on eIF2α phosphorylation, which leads to global translational attenuation with concomitant upregulation of select protein coding transcripts, such as ATF4, as a homeostatic response to certain stimuli, chronic or extreme stress can augment the signaling pathways downstream of eIF2α phosphorylation and result in maladaptive responses and even cell death.

Canonical ATF4 signaling includes induction of cytotoxic signals, such as CHOP upregulation, under conditions of unresolved stress and prolonged eIF2α phosphorylation, leading to cell death (3). Furthermore, sustained translational attenuation by itself is disruptive to neuronal function on several levels (41, 42, 59, 60). Formation of stress granules and liquid droplet aggregates, such as that seen in certain neurodegenerative conditions, has been shown to be dependent on elevated eIF2α phosphorylation as it leads to the accumulation of mRNAs (61, 62). Neurons also suffer from translation inhibition due to the fact that synapses turn over large amounts of proteins, and synaptic cell-to-cell transport/communication is critical to the function of neuronal networks. A large body of research highlights the necessity of translation, and the related bidirectional role of eIF2α phosphorylation, in long-term memory potentiation (LTP) and synaptic plasticity (63–66). Specifically, mice heterozygous for an alanine mutation at serine 51 of eIF2α exhibited enhanced LTP and memory consolidation, whereas pharmacological induction of eIF2α phosphorylation had the reverse effect (67). Additionally, pharmacological downregulation of ATF4 in mice resulted in enhanced synaptic plasticity and long-term memory (68). Treatment with a nonspecific, general translation inhibitor, anisomycin, however, did not lead to differences in LTP (69). Mice knocked out for *EIF2AK4* or *EIF2AK2*, the genes encoding GCN2 or PKR also exhibited enhanced memory consolidation (70, 71), whereas forebrain-specific ablation of *EIF2AK3* in adult mice resulted in impairment of cognitive and information processing (72). These signaling pathways and outcomes of prolonged eIF2α phosphorylation might partially explain the many neurodegenerative phenotypes with which it is associated, such as aggregate-associated pathology, synaptic dysfunction and loss, neuronal death, and memory deficits (2, 40, 49).

Several studies have also demonstrated that sustained eIF2α phosphorylation is correlated with cognitive deficits in neurodegenerative disease models. For example, translation, synaptic deficits, and neuronal loss were rescued by overexpression of GADD34 in a mouse model of prion disease (60). Intriguingly, GADD34 was the only factor downstream of CHOP that was not upregulated during disease progression in this disease model (60). Interestingly, *DDIT3* knockout ameliorated cell death in a mouse model of PD (73). Additionally, an eIF2α siRNA protected against cytotoxicity induced by rotenone, a mitochondrial inhibitor used to model PD by inducing oxidative stress resulting in phosphorylation of PERK, PKR, and eIF2α as well as cell death (74). While these are common effects of all ISR kinases,

each kinase also induces its own specific downstream signaling pathway and has distinct pursuant implications in neurodegeneration.

## PKR-Like ER Kinase

PERK is 1 branch of the UPR, activation of which is one of the most pervasive observations across neurodegenerative diseases. Specifically, eIF2α phosphorylation attributed to PERK has been observed in the postmortem brains, and sometimes the cerebrospinal fluid (CSF), of clinical patients with AD, PD, ALS, HD, progressive supranuclear palsy (PSP), and HIV-associated neurocognitive disorders (HAND), a neurodegenerative condition which persists in up to 50% of patients on antiretroviral therapy (ART) with suppressed virus (38, 41, 59, 75–78). Thus, misfolded proteins such as amyloid beta (Aβ), α-synuclein, and PrP, which are major hallmarks of many neurodegenerative diseases, may act in a feed-forward mechanism, inducing chronic PERK signaling via the UPR. Akin to the ISR, a large body of evidence suggests that the UPR can switch from an adaptive/proteostatic profile to maladaptive/cytotoxic signaling in response to severe or prolonged stress such as that seen in neurodegenerative pathologies (38, 41, 49, 51, 53). While this switch is believed to be dependent on the context and strength of the stress, the precise mechanism underlying this protective/toxic switch remains an ongoing question in the field.

The PERK arm of the UPR has the most well-established connections to maladaptive processes and has even been suggested to act as an adaptive signaling pathway only in coordination with the other arms of the UPR (4, 40, 49, 50, 59, 79, 80). In fact, IRE1 activity results in increased p58 levels, which inhibit PERK, suggesting the presence of tight regulation aimed to prevent overactivation (81). Additionally, PERK is the only branch of the UPR that remains active under chronic stress conditions when the UPR is presumably in the maladaptive phase (82). Many studies support the direct role of PERK dysregulation in elevated eIF2α phosphorylation in neurodegeneration (41, 83). PERK signaling is upregulated early in neurodegenerative disorders such as AD and PD, as shown in animal models and human specimens (84–94). Moreover, genetic ablation of *EIF2AK3* in the central nervous system (CNS) was shown to be protective in prion-inoculated mice and in a mouse model of AD (91, 94). Increased eIF2α phosphorylation was also observed in postmortem cortex of patients with HAND compared to neurocognitively normal individuals (95). Additionally, a recent transcriptome analysis by Venkatachari et al (96) identified *EIF2AK3* among the genes associated with neurodegeneration in HIV-infected males.

Several recent findings on PERK haplotypes provide further evidence for dysregulated PERK activity and elevated eIF2α phosphorylation levels as contributors to neurodegeneration. Of the 5 known PERK haplotypes, the most prominent are haplotypes A and B, which are present in 67.6% and 31.1% of the general population, respectively (97). Four single-nucleotide polymorphisms differentiate these 2 major haplotypes; 3 of which result in changes in amino acids, 2 of which are within the luminal domain and 1 of which is within the kinase domain of PERK (98). Intriguingly, experiments in cultured lymphocytes from homozygous donors for either haplotype showed that the B haplotype of PERK led to higher eIF2α phosphorylation levels in response to the ER stress inducer thapsigargin, compared with the A haplotype (97). This enhanced response is likely the result of the amino acid changes conferring differences in PERK activation, activity, and/or downregulation between the 2 haplotypes. As noted, eIF2α phosphorylation level is a potential rheostat for downstream signaling pathways. Thus, a cell with more active PERK, such as that might be the case with PERK haplotype B, is expected to be biased toward maladaptive signaling. Conversely, 1 study in a tauopathy model for PSP reported that PERK haplotype B was a hypomorph at low stress levels, which also led to maladaptive outcomes due to ER stress sensitivity (99). Nonetheless, PERK haplotype B has been identified as a risk factor for AD and PSP, a neurodegenerative tauopathy (78, 100–105). This risk factor status makes it a compelling target for pharmacogenetic studies, particularly for the development of personalized medicine in these at-risk populations.

Despite all these disease implications, it remains that PERK signaling acts as an adaptive response during acute ER stress and plays numerous physiological and prosurvival roles, as outlined above (79, 106, 107). In following, many studies have convincingly showed that ablation of PERK signaling was toxic and that augmentation of eIF2α phosphorylation conferred protection from cell death (38, 59). For example, genetic ablation of *EIF2AK3*, resulted in increased sensitivity to cell death following stress induction, which was expected due to the prevention of the initial adaptive phase and ablation of PERK before stress onset instead of during chronic stress (106). In another study, allicin, a garlic extract, was used to upregulate the antioxidant pathway via PERK phosphorylation of NRF2, which ameliorated ER stress-mediated cognitive deficits in rats (108, 109). As previously noted, functionally null mutations in *EIF2AK3*, lead to Wolcott-Rallison syndrome which characterized by early neurodegeneration (110), which is expected based on the previously described role of PERK in neuronal wiring. The extrinsic morphogenesis factor semaphorin-3A induces acute local translation of proteins involved in proteostasis and the actin skeleton, which leads to PERK activation concurrent with ERK1/2-PP1-mediated dephosphorylation of eIF2B that increases its GEF activity and overcomes the translational block, illustrating the role of this signaling pathway in axon guidance and branching (111). Furthermore, beyond its contribution to protein synthesis-dependent memory consolidation, PERK signaling is implicated in cellular calcium dynamic-dependent working memory, which was found to be impaired in mice with pharmacologically inhibited PERK or in brain-specific *EIF2AK3*, knockout mice (71).

PERK signaling is also important to a concept known as hormesis, whereby a constant state of nonlethal ER stress preconditions and provides protection of cells against further stresses (41). This is very important for cells that synthesize and secrete large amounts of proteins, such as pancreatic β cells, lymphocytes, and oligodendrocytes. Hormesis is achieved by upregulation of the PP1 phosphatase cofactor

GADD34, which targets PP1 to phosphorylated eIF2α (41). A study, in which eIF2α phosphorylation levels were increased artificially by PERK activation independent of stress, demonstrated that this effect was enhanced by upregulation of GADD34, which persisted after treatment and mediated future protection (112). These beneficial roles of PERK highlight that studies elucidating the role of PERK in neurodegeneration must be cautious of its dichotomous roles.

Additional studies and reviews have proposed PERK as decisionmaker in cellular fate (2), with outcomes depending on stress levels and the interaction of PERK with various modulators (79, 80). One study, using selenite-induced PERK activation, showed that phosphorylated PERK facilitated p38 dissociation from the inhibitory Hsp90-PERK complex such that it could be activated by autophosphorylation. Additionally, phosphorylated p38 induced p53 phosphorylation and activation, which in turn induced eIF2α phosphorylation and eIF4E dephosphorylation. Thus, ATF4 association with the *DDIT3*, promotor was enhanced and association with the *MAP1LC3B*, the gene encoding LC3, promotor suppressed, thereby promoting apoptosis by attenuating autophagy (80, 113). Furthermore, autophagy and pursuant lysosomal degradation are frequently reported to be impaired in neurodegenerative diseases, which might partially explain the predominance of maladaptive outcomes during PERK activation in these conditions (57, 93, 114, 115). Conversely, a study on cancer highlighted that PERK mutants reported in the Human Cancer Genome Atlas were consistently hypomorphic and demonstrated that the *EIF2AK3* levels dictated the tumor suppressive versus the oncogenic properties of PERK via pharmacological and genetical modulation (106). Thus, the opposing effects of its activity lend further support for PERK as an ideal candidate for regulating the aforementioned switch between homeostatic and proapoptotic roles.

One final aspect that is commonly overlooked in evaluating PERK and other kinases of the ISR during neurodegenerative processes is that neurons do not exist in isolation in vivo. Any insult triggering ISR in the CNS will also result in ISR activation in glial cells, which are resistant to ER stress-mediated cell death. Instead, astrocytes produce cytokines as part of an innate immune response (116); these cytokines create a neuroinflammatory environment which, over time, can lead to neuronal dysfunction both directly and by inducing ER stress in the neurons themselves. PERK signaling, specifically, has been linked to the induction of interleukin (IL)-6, C-C motif chemokine ligand (CCL)-2, and CCL-20 expression in astrocytes (116). Sustained eIF2α phosphorylation was necessary for the cytokine induction via PERK signaling, whereas ablation of the PERK signaling by genetic haploinsufficiency was shown to attenuate the expression of cytokines (117).

## Protein Kinase R

Given its ability to be activated by inflammatory cytokines, ER stress, and oxidative stress in addition to the canonical viral infection, it is unsurprising that PKR activation is observed during various stages of many clinical neurodegenerative conditions. PKR's ability to dictate cell fate and its function as a proapoptotic kinase and an inducer of downstream inflammatory cascades support a role for PKR activation in disease pathogenesis. Furthermore, besides eIF2α, PKR has several substrates implicated in neurodegeneration (25, 118, 119). For example, NF-kB activation downstream of IKK interaction results in the production of several cytokines widely believed to be pathogenic in neurodegenerative as well as apoptotic pathways.

Several studies in in vitro and animal models and patient samples suggest that PKR may play a key role in AD pathogenesis: In these various AD-related contexts, phospho-PKR (p-PKR) and p-eIF2α are elevated and correlate with cognitive decline (84, 114, 120–123). In particular, 1 study showed that extracellular Aβ led to PKR-dependent induction of neuronal apoptosis that involved eIF2α phosphorylation without UPR activation (118, 124). Additionally, PKR phosphorylation was reduced in postmortem brain tissue of patients who received Aβ immunotherapy for AD compared to untreated AD patients (125). In that study, p-PKR levels correlated with Aβ load and markers of neurodegeneration. The authors also observed a decrease in microglial activation, while what microglial activity remained correlated positively with PKR phosphorylation, suggesting a role for PKR in the induction of microglia-associated inflammatory response (125). Another study using Aβ treatment of SH-SY5Y neuroblastoma cells in vitro and in $APP_{SL}PS1$ knock-in mice demonstrated increased levels of p-PKR and the proapoptotic protein FADD in parallel with caspase-3 and caspase-8 activation, all of which were blocked with a PKR inhibitor (118, 126). Relatedly, PKR activation was reported in both brain tissue and peripheral blood mononuclear cells isolated from patients with AD (119, 126); PKR inhibition in these cells decreased the production of several proinflammatory cytokines and inhibited caspase-3 activation, encouraging further investigation of the role of peripheral cells in neuropathogenesis. In multiple models, PKR was also shown to directly bind and phosphorylate α-synuclein, whereas pharmacological inhibition or silencing of PKR was shown to alleviate cellular degeneration in correlation with reduced α-synuclein phosphorylation, implicating a role of PKR activation in PD and similar synucleinopathies (127). Increased p-PKR levels were observed in hippocampal tissue specimens of patients with PD and HD, suggesting an association of PKR activation with extrastriata degeneration (128). Finally, intracytoplasmic PrP is proposed to activate PKR: p-PKR levels were reported to correlate with neuronal apoptosis, microglia activation, and markers of astrocytosis as well as disease severity in samples from patients with Creutzfeldt-Jakob disease (129).

Interestingly, *EIF2AK2*, was identified as one of the factors associated with neurodegeneration in the transcriptome study of HIV-infected men by Venkatachari et al (96). In another study, treatment of neuroglial cultures with the HIV-1 surface protein gp120, which can induce neuronal damage and death, resulted in increased PKR protein levels in both primary rat neurons and astrocytes, with PKR activation observed only in neurons similar to that seen in patients with HIV-associated dementia, the most severe form of HAND (130). The authors also showed that pharmacological PKR inhibition and genetic *EIF2AK2* knockout were neuroprotective in the same experimental paradigm. Another study examined the role of PKR in

viral infections in the context of a prion model of neurodegeneration; to that end, the authors mimicked the inflammatory response to systemic viral infections using toll-like receptor 3 to induce type 1 interferons and observed an accelerated disease progression accompanied with increased apoptosis, which correlated with *EIF2AK2* and *FAS* transcription (131); the authors inferred that neurodegeneration sensitized the CNS to exaggerated inflammatory responses by microglia, supporting the role of inflammation in exacerbating neurodegeneration.

## General Control Nonderepressible 2

GCN2 is prominently expressed in the brain and is a known regulator of synaptic plasticity, learning, and memory in the hippocampus, as demonstrated in a study where knockout of *EIF2AK4* resulted in altered LTP profiles in response to electrical stimuli. These results were correlated with memory-related behavioral deficits, and, as expected, reduced ATF4 and increased CREB expression (70). In a separate study linking ribosomal stalling, which can be triggered by oxidative stress, to neurodegeneration, GCN2 activation, eIF2α phosphorylation, and induction of ATF4-regulated genes were seen as intermediate steps (132). However, in the model used in that study, which utilized mutations in translational factors to promote ribosome stalling, the GCN2-ATF4 signaling appeared to be neuroprotective. Still, given that the type of stress that induces the ISR can dictate the outcome, GCN2 is likely to play a role in chronic neurodegenerative pathologies, although studies investigating GCN2 in this context are limited.

In 1 study, amino acid deprivation-induced GCN2 activity was shown to result in the ATF4-dependent upregulation of presenilin-1, the catalytic component of γ-secretase (133). Presenilin-1 is involved in amyloid precursor protein (APP) processing, including the generation of both soluble APP and Aβ peptide from APP, implicating GCN2 activity in AD and other conditions with Aβ pathology. As GCN2 can also be activated in response to oxidative stress, GCN2-presinilin signaling may also constitute a feed-forward pathway playing a role in the pathogenesis of progressive neurodegenerative conditions. For example, a study using okadaic acid treatment to model AD in neurons demonstrated GCN2 activation (84). Additionally, 1 study utilizing mouse models of familial AD observed that the deletion of GCN2 rescued synaptic plasticity and spatial memory (91). GCN2 is also activated in response to viral infections and was previously described to interact directly with HIV, raising the possibility of its involvement in virus-related neurodegenerative conditions such as HAND.

## Heme-Regulated Inhibitor

HRI is not thought to play a significant role in neuronal p-eIF2α levels given that it is primarily expressed in erythroid cells and has no known physiological roles in other cell types. Albeit severely understudied, it is clear that the roles *EIF2AK1* plays in cell types that interact with neurons have potential implications in neurodegeneration. For example, knockdown of HRI in macrophages, which express HRI, was shown to result in defects in macrophage maturation and inflammatory responses to LPS (23). The same study also highlighted the role of macrophages in recycling iron from senescent red blood cells, a process inhibited by the upregulation of hepcidin under conditions of inflammation and oxidative stress. In the *EIF2AK1* knockout mice, hepcidin levels were reduced in both control and LPS-treated animals, potentially due to decreased cytokine production, leading to increased serum iron levels and impairing erythropoiesis. (23). Intriguingly, dysregulated iron homeostasis has been extensively implicated in neurodegeneration (134, 135). Particularly, the disruption of iron homeostasis in microglia and its contribution to chronic inflammation mediated by microglia were reported previously (136). Whether HRI signaling plays a role in microglia akin to that reported in macrophages remains unclear.

In Schwann cells, disruption of mitochondrial activity, a common hallmark of neurodegenerative diseases, was shown to induce a maladaptive ISR (137). Specifically, HRI is exclusively activated by this noncanonical stress, which leads to the remodeling of lipid metabolism to downregulate fatty acid synthesis and upregulate β-oxidation. This results in decreases in myelin components, which likely contributes to pathological demyelination, as well as accumulation and release of acylcarnitines, which can cause axonal degeneration via disruption of the calcium homeostasis. A study on oligodendrocytes also reported that mitochondrial deficits led to changes in metabolism in the form of increased reliance on glycolysis; however, the shift was sufficient to meet the energy needs of the cells, and no demyelination or axonal degeneration was observed (138). The relevance of these studies and the need for further investigations are highlighted by the observations that energy depletion/metabolism remodeling, fatty acid oxidation, and altered calcium homeostasis are commonly observed, and are believed to play a role, in neurodegenerative conditions.

## ISR Kinase Crosstalk

Importantly, studies in neurodegenerative disease often attribute the outcomes of manipulating eIF2α phosphorylation levels to the signaling events related to the specific ISR kinase that is most directly impacted within the disease model in question. However, there is a broad and continually expanding overlap among the ISR kinases beyond their canonical activation schemes, as previously highlighted. Many of these studies do not consider and/or control for contributions of the other kinases in the family. Furthermore, even for those studies that account for potential crosstalk, concerns remain regarding the specificity of many of the commonly employed tools to manipulate these kinases. For example, in a study where the inflammatory molecule 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 was shown to activate multiple eIF2α kinases, potentially partially and not exclusively through proteasomal inhibition, the investigators attempted to identify the specific responsive kinases (139). However, they found that thapsigargin, considered to be a PERK inducer, also activated PKR and that PKR inhibitors inhibited PERK as well. In the same study, a PERK inhibitor reduced stress granules in response to sodium selenite, which was traditionally considered to only activate HRI. Addi-

tionally, it is likely that in addition to the presence of a cross-talk among the 4 ISR signaling branches, inhibition of one of the kinases can induce the ISR, which in turn stimulates another ISR kinase in a feed-forward cascade. Going forward, it will be pertinent for the field to examine whether activation of the ISR during neurodegeneration initiates in response to a specific stress, such as ER stress, oxidative stress, or inflammation, which in turn tends to drive the others, or if different diseases initiate different stresses, and/or if the various branches of the ISR are triggered concomitantly in pathology. Similarly, it will be illuminating to examine whether there are compensatory mechanisms among the ISR kinases. Furthermore, whether the order of induction of ISR kinases is dependent on disease and/or whether they are indeed activated independently in disease will shed a significant light in elucidating the complex network of ISR kinase-associated signaling.

## MALADAPTIVE OUTCOMES OF eIF2α PHOSPHORYLATION

The consequences of increased ISR kinase activity have largely been focused on apoptosis as a mechanism of neurodegeneration and memory impairments due to inhibition of general protein translation. However, there are multiple novel pathways that are being explored as mechanisms downstream of ISR kinase activation, mainly PERK and PKR, that potentially contribute to axonal damage, neurodegeneration, and the consequent cognitive impairment. Amyloid and tau pathology, as well as repeat-associated non-AUG (RAN) translation and neuroinflammation, have been linked to activation and/or dysregulation of ISR kinases and altering the activity of ISR kinases appears to modulate these distinct pathological pathways. In this section, we review the evidence linking ISR kinases with downstream detrimental mechanisms observed in different neurodegenerative diseases.

### Repeat-Associated Non-AUG Translation

Neurodegenerative diseases such as HD, ALS, and FTD are included in a class of disorders known as microsatellite expansion disorders, due to their association with such mutations (140–142). Specifically, hexanucleotide repeat expansions in *C9ORF72* and trinucleotide repeat expansions in *HTT* have been extensively linked to the pathology of these diseases, and have recently been demonstrated to undergo RAN translation (143–146). RAN translation refers to translation which occurs without the involvement of the canonical AUG start codon and, intriguingly, has been demonstrated to be intertwined with ISR signaling.

RAN translation was first observed in the context of spinocerebellar ataxia type 8 (SCA8), in a study where transfection of cells with *ATXN8* containing a CAG repeat expansion, which is a microsatellite mutation associated with the SCA8, resulted in the production of polyglutamine proteins, even when the *ATXN8* start codon was mutated (147). The investigators also found that this noncanonical translation could initiate in all 3 reading frames, producing polyalanine and polyserine proteins in addition to the clinically observed polyglutamine (147). The existence of these alternative RAN translation products in clinical disease was validated by positive staining in the brains of SCA8 patients for polyalanine (147). Furthermore, the investigators also validated, by staining, the existence of another alternative RAN translation product, polyglutamine, in the brains of myotonic dystrophy 1 and 2 patients, and found polyglutamine staining associated with caspase 8 staining, suggesting the ability of these proteins to induce apoptosis (147). Indeed, staining of multiple RAN products in HD-affected brains colocalized with neuronal loss, microglial activation, and apoptosis, and many more studies have pointed toward to the toxicity of a continually growing list of RAN products (140, 141, 143, 148).

Since its identification, RAN translation has been determined to require hairpin formation, a common feature of nucleotide repeat expansions, and to have some degree of length dependence, while follow-up studies have further shown it to be capable of bidirectional translation, utilizing both the sense and antisense transcripts (147, 149–151). While the precise mechanisms of RAN translation remain largely ambiguous, it has been proposed to operate similarly to IRES translation, given the dependence on RNA hairpins, which may create a blockade for scanning ribosomes and facilitate noncanonical initiation in the context p-eIF2α-induced limited preinitiation complex availability (147, 152–155). Controversially, different studies have suggested both cap-dependent and cap-independent mechanisms regarding RAN translation of different expansions in different diseases. Specifically, 1 group showed RAN translation of the *C9ORF72* hexanucleotide expansion associated with ALS and FTD was cap-dependent, and selectively enhanced by canonical ISR activation, utilizing a near cognate CUG start codon during p-eIF2α-dependent alteration in start codon fidelity (156). Separately, ISR activation by pharmacological induction, glutamate mediated toxicity, and optogenetically altered neuronal excitation were all shown to drive RAN translation in primary cortical neurons and ALS-patient-derived spinal motor neurons (157). Thus, RAN is a novel, specifically upregulated translation mechanism downstream of eIF2α phosphorylation, with potentially maladaptive outcomes.

However, it should be noted that while Green et al (156) made these observations across multiple cell types, using multiple methods and transfections to support the same conclusion, it also utilized linear mRNA repeat constructs, which may be processed differently than when they are in their physiological intronic context. The study further demonstrated that the produced dipeptide repeat proteins themselves also triggered the ISR and induced stress granules, which could constitute a feed-forward mechanism of toxicity in pathology (156). Similarly, another study linked RAN translation of a *C9ORF72* expansion to the use of a CUG start codon and IRES-like mechanisms and described the ISR-mediated enhancement of RAN translation as well as dipeptide repeat protein-mediated induction of the ISR (158). However, that study also identified dependence on the noncanonical initiation factor eIF2A for RAN translation and noted that RAN translation persisted during the ISR-mediated decline in cap-dependent translation (158). Green et al (156) made reference to RAN-translated proteins in which no near cognate start codons have been identified, suggesting that different repeats may have distinct RAN initiation mechanisms.

In accordance, another study, utilizing HeLa cells stably expressing various translation reporters, published on cap-independent RAN translation of the *C9ORF72* hexanucleotide repeat expansion from the endogenously spliced first intron, but only after its translocation to the cytoplasm (159). The study showed that this cap-independent RAN translation was also upregulated in response to ISR stressors, and was dependent on eIF2α phosphorylation, which was necessary and sufficient for the promotion of RAN translation (159). In addition, the investigators found that upon expression the prion-like domain of TDP-43 mislocalized to the cytoplasm, formed inclusions, and induced stress granule formation, eIF2α phosphorylation, and RAN translation of *C9ORF72* expansions (159).

TDP-43 as well as several other RNA-binding proteins (RBPs) are frequently mutated, and commonly mislocalized and/or aggregated in ALS and FTD, including in cases involving *C9ORF72* expansions; thus, this may constitute another feed-forward mechanism of disease pathogenesis (142). However, a separate study found that the expression of wild-type human disease-associated RBPs, including TDP-43, FUS, and hnRNPA2B1, resulted in their binding to and alteration of the structure of a SCA type 31-associated pentanucleotide repeat expansion (160). This interaction was shown to be direct and suppressed toxicity, specifically eye degeneration in *Drosophila*, by acting as an RNA chaperone, dispersing downstream, clinically observed RNA foci and diminishing RAN-mediated pentapeptide production (160). Furthermore, RNAi knockdown of endogenous TDP-43 exacerbated eye degeneration (160). On the other hand, short repeat peptides were found to ameliorate toxic TDP-43 expression, indicating that there is intricate crosstalk required for ribonucleoprotein homeostasis which can be unbalanced from either side in pathology, and suggesting a possible physiological function for such repeats (160). In summary, several aspects of the RBPs implicated in neurodegeneration, including their disordered, misfolding prone domains, their common sequestration in stress granules, and the emerging evidence of the intricate, seemingly bidirectional crosstalk between them, ISR signaling, and RAN translation, suggest that therapeutically modulating them will, in effect, target a stress signaling intersection that may beneficially combat multiple drivers of pathology simultaneously. For example, targeting RNA folding homeostasis has preliminarily demonstrated therapeutic benefit. Indeed, RNA helicase DDX3X was identified as a repressor of RAN translation in a CRISPR screen (161). Additionally, targeting repeat expansions with antisense oligonucleotides has been a promising area of therapeutic development (140, 141, 161). Furthermore, the discovery of the bidirectional capacity of RAN translation suggests that further development of such efforts to target both strands may enhance therapeutic benefit.

The pathogenesis of microsatellite expansion disorders was classically presumed to be driven by a mixture of RNA gain- and loss-of-function mechanisms, in accordance with the observation of RNA foci and aggregates in postmortem diseased tissue, as well as the toxicity of the corresponding homopolymeric expansion proteins, which are also known to be aggregated in diseased brains (140, 142). The discovery of RAN translation overturned the traditional thinking and expanded the possible players of pathogenesis to include not only proteins encoded by alternate reading frames but also those which could be produced from intronic and noncoding repeat expansions. The field of RAN translation is a fairly new and still rapidly evolving area of research with many questions still unresolved, as evidenced by the continually emerging plethora of reviews and perspectives (140–142, 148). A recent high throughput screen identified 5 diverse, bioactive small molecules which were able to dose-dependently inhibit RAN translation of repeats associated with ALS, FTD, and fragile X-associated tremor ataxia syndrome (162). Importantly, while the molecules were shown to interact with different aspects of RAN translation, the mechanisms of some were elusive, highlighting the gaps in our understanding of RAN translation (162). Furthermore, while none of the molecules drastically affected canonical translation, proving selective targeting of RAN translation to be feasible, limited investigation still suggested that these molecules were toxic to cells (162). Thus, while emerging interventions such as antibodies targeting RAN proteins are theoretically promising, significantly better understanding of the mechanisms of RAN induction and identification of toxic contributors in the context of various repeat expansions are desperately needed if RAN is to be successfully targeted for therapeutic purposes. Further, understanding the potential interplay between ISR translation attenuation and RAN translation in microsatellite expansion disorders will be critical for effective treatment.

## BACE1 Upregulation and Amyloidogenesis

Amyloidogenesis is another possible downstream consequence of chronic ISR kinase dysregulation that might mechanistically contribute to neurodegeneration. The amyloidogenic pathway of AD, which results in the generation and accumulation of toxic Aβ species, is initiated by the cleavage of APP by the protease β-site APP cleaving enzyme-1 (BACE1). BACE1 protein levels are increased in clinical AD and HAND in vivo as well as in response to excitotoxic injury and ARTs in vitro (163–168). Additionally, BACE1 mediates Aβ deposition and neuronal loss in mouse models of AD, underlining its importance in neurodegeneration (169). Studies exploring the link between ISR and BACE1 in AD demonstrated that p-eIF2α levels correlated with BACE1 protein levels in the temporal and frontal cortices and with Aβ in the frontal cortex (170). Interestingly, the transcript of BACE1 was identified in a subset of mRNAs that were induced in response to pharmacological induction of eIF2α phosphorylation in both BACE1-overexpressing HEK293 cells and, more importantly, in primary neurons, underlying the possibility that inducing eIF2α phosphorylation might be sufficient for the upregulation of BACE1 protein levels in neurons (171). Furthermore, a study using ethanol treatment of the human-derived neuroblastoma cell line SK-N-MC concluded that increased p-eIF2α led to upregulation of BACE1 protein levels and Aβ secretion through the stimulation of cyclooxygenase-2 and prostaglandin 2 production (172). These studies suggest that activating the ISR might have detrimental effects via the amyloidogenic pathway. The observed increase in BACE1 levels was shown to consequently lead to a significant increase

in Aβ production in primary neurons and a trend toward increased amyloidogenesis in the Tg2576 mouse model of AD. Therefore, in the presence of stress conditions, increased levels of p-eIF2α can potentially lead to Aβ production and aggregation through BACE1 upregulation. Additional studies identified PERK as the kinase mediating the BACE1 upregulation in mouse models of AD. Similarly, 5XFAD mice, which carry mutations in the *APP* and *PSEN-1* genes and have advanced amyloid pathology, were reported to have increased levels of p-eIF2α as well as phosphorylated PERK (88, 173). Furthermore, crossing PERK haploinsufficient mice with 5XFAD mice resulted in reversal of increased BACE1 levels and amelioration of Aβ plaque burden. These findings were recapitulated in other contexts as well. For example, upregulation of BACE1 by certain antiretroviral drugs was shown to be mediated by PERK, suggesting its critical role in mediating an increase in BACE1 levels and potentially amyloidogenesis of HIV-infected individuals (167).

Other ISR kinases have also been shown to be involved in regulating BACE1 expression. Particularly, p-PKR levels were increased in AD patients, and CSF p-PKR levels correlated with cognitive decline in AD patients (120, 174–178). In 1 study, activation of PKR and consequent eIF2α phosphorylation led to derepression of BACE1 expression in herpes simplex virus type 1-infected neuron-like SH-SY5Y cells (174). Similarly, inhibition of PKR attenuated BACE1 protein levels that were increased in response to oxidative stress in SH-SY5Y cells and in LPS-tread mice (170, 179). One recent study elucidated the link between PKR and BACE1: In a mouse model utilizing thiamine deficiency to recapitulate oxidative stress in AD, activation of the PKR-p-eIF2α axis and increased levels of BACE1 and Aβ were observed in the thalamus, effects which were blocked by knocking out *EIF2AK2* or treatment with a PKR inhibitor (180). In a similar manner, a study found that crossing *EIF2AK2* knockout mice with the 5XFAD mouse model led to improved memory consolidation (181); Aβ accumulation and BACE1 activity were significantly decreased in the double-mutant mice, indicating that PKR modulated amyloidogenesis in vivo. HRI has also been mechanistically linked with BACE1 induction. Although few studies reported the presence of HRI in the brain, 1 study found HRI mRNA and protein in both human and mouse hippocampus; interestingly, HRI colocalized with synaptic spines (182). Further examination revealed that inhibiting HRI blocked nitric oxide-induced BACE1 upregulation in SH-SY5Y cells. These mechanisms could potentially be linked to AD pathology and other neurodegenerative diseases that are characterized by Aβ aggregates.

Although these studies suggest that ISR kinase activation and consequent p-eIF2α lead to increases in BACE1 protein levels, another study found that GCN2 deletion in 5XFAD mice further exacerbated BACE1 levels, which was due to the compensatory overactivation of the PERK pathway with a consequent increase in p-eIF2α levels (183). Additionally, as addressed previously, Aβ aggregates might directly activate 1 or more of the ISR kinases to exacerbate neurodegeneration via a positive feedback loop (184).

While the above findings highlight the ISR kinase-p-eIF2α-BACE1 pathway as an important therapeutic target, BACE1 inhibitors have unfortunately been largely disappointing in AD clinical trials (185). For example, a phase III clinical trial (EPOCH) of verubecestat, the first BACE1 inhibitor to reach late-stage clinical trials in AD patients, was recently terminated early due to observed adverse effects (186, 187). This is a common theme as inhibition of BACE1 has proven challenging and questionable in terms of safety, presumably due to mechanism-based side effects (188, 189). It is speculated that BACE1 inhibitors might be failing for a combination of reasons: inhibition of the beneficial roles of BACE1, such as important functions at the synapse (188, 190); administration too late in neuropathological progression; stabilization of BACE1 (191). Additionally, BACE2 is largely nonneuronal, but highly homologous to BACE1, and thus some of the detrimental effects of BACE1 inhibition may be accounted for by the off-target effects on BACE2, as all thus far tested molecules have not been selective between the 2 isoforms (185, 188, 192). Despite these failures, ongoing research has been aimed at resolving these issues related to BACE1 inhibition. In fact, AM-6494, a cyclopropylthiazine molecule recently discovered by structure-based design, demonstrated specificity for BACE1 over BACE2 in an in vitro assay, passed a 14-day toxicity screen in rats and dogs, and proved to be able to reduce CSF Aβ in rats and rhesus monkeys (193). Additionally, phage-display and biopanning were recently used to identify a 12-mer peptide which tightly bound BACE1, with selectivity over BACE2, prevented the cleavage of APP in in vitro assays and produced no detrimental morphological changes when administered to SHY5Y cells (194). While it remains to be seen if such competitive peptide inhibitors can be fine-tuned to target the BACE1-APP interaction over other BACE1 substrates, these recent developments highlight that there is still therapeutic potential for BACE1 inhibition.

Still, while off-target effects may explain the toxicity of previously evaluated BACE1 inhibitors, and thus allow for improvement and refinement, the failure of these drugs to provide clinical and cognitive benefit in disease contexts, even when efficaciously reducing CSF Aβ levels, highlights the need to critically examine the amyloid hypothesis and assess whether targeting amyloidogenesis is a viable therapeutic option, particularly in isolation (185, 189). For the development of BACE1-targeting drugs to be successful in clinical trials, 2 important issues should be resolved. First, a better understanding of the connection between Aβ accumulation and cognitive decline is necessary to prioritize the administration of these drugs to amenable cases. Similarly, the physiological functions of BACE1 should be elucidated further to improve the selectivity of the drugs. It is possible that inhibiting chronic ISR activation upstream of amyloidogenic processing may encounter similar obstacles as those observed with BACE1 inhibitors. Thus, inhibition of ISR to treat neurological disease should be optimized accordingly to avoid the negative consequences observed with BACE1 inhibitors.

## Tau

Integrated stress response kinases and activation of the eIF2α pathway are extensively associated with a range of clinical diseases termed tauopathies, which are characterized by

the presence of neurofibrillary tangles. Phosphorylation of tau, a microtubule associated protein, at 1 or more epitopes leads to its aggregation into insoluble tangles, which correlate with cognitive decline in AD (195). Evidence suggests that ISR kinases might be mediating tau pathology in tauopathies, including AD and PSP. First, both PERK and PKR are activated in clinical PSP and AD (85, 90, 92, 114, 196). Similarly, increased p-PERK expression was observed in neurons and glia in the tissue specimens of patients with frontotemporal lobar degeneration and early tau pathology in the hippocampus, while markers of UPR activation correlated with phospho-tau in AD patient tissue (92). Activation of PERK and PKR was reported to closely correlate with levels of phosphorylated tau, observed primarily in early pretangle neurons as well as in astrocytes and oligodendrocytes in AD tissue (197). In the hippocampus of AD, one of the most severely affected areas in disease, p-PERK colocalized with AT8, a marker of hyperphosphorylated tau, in neurons (198). Furthermore, a genome-wide association study reported that *EIF2AK3* was a genetic risk factor for PSP (103). These findings all together suggest ISR dysregulation as an underlying mechanism of pathology and/or a consequence of aberrant tau protein aggregation in tauopathies. However, mechanistic studies provide evidence for ISR kinases as upstream mediators of tau pathology. For example, using the ER stress inducers tunicamycin and thapsigargin, which activate the UPR and PERK as a consequence, albeit by different mechanisms, several studies observed that chemically induced ER stress was sufficient to increase the phosphorylated tau levels (199–201). Pharmacological inhibition of PERK reduced the levels of phosphorylated tau and alleviated cell death in the rTg4510 mouse model of tauopathy, wherein mice harboring the P301L tau mutation exhibit pathological findings mimicking AD and FTD (202). A possible mechanism underlying this finding is alteration in the levels and/or activation of tau kinases such as cyclin-dependent kinase 5 and glycogen-synthase kinase-3 beta (GSK-3β). Several studies found that ER stress as well as specific PERK activation induced GSK-3β activity (115, 198, 203, 204). Activation of tau kinases leads to tau hyperphosphorylation, which affects microtubule-binding properties and facilitates aggregation; tau aggregation might also be mediated by p-eIF2α, which was found to be elevated and colocalized with phosphorylated tau in the brains of patients with sporadic AD (114). Several studies implicate PKR in tau pathology as well. Specifically, inhibiting PKR either pharmacologically or via siRNA inhibited tunicamycin-induced tau phosphorylation in SH-SY5Y cells, suggesting that PKR might also be upstream of tau pathology in the context of disease (198).

As mentioned previously, PERK haplotype B is associated with AD and PSP, 2 neurodegenerative diseases characterized by neurofibrillary tangles composed of tau proteins. PERK haplotype B was found to be associated with increased PERK activation in the brains of PSP cases, suggesting that PERK coding variants associated with haplotype B lead to the chronic activation of the PERK pathway and subsequent detrimental outcomes (196). This mechanism is in agreement with studies showing that PERK haplotype B has increased kinase activity and responds more robustly to ER stress compared to PERK haplotype A (97). It remains unclear exactly how PERK haplotype B and higher PERK activity may lead to increased risk of developing tauopathies. Possibly, patients homozygous for PERK haplotype B have increased kinase activity, enhanced chronic ISR response, and consequently increased tau kinase activation. In turn, this may lead to increased tau phosphorylation and aggregation. Contradicting earlier studies, Yuan et al (99) found that PERK haplotype B was a hypomorph in neurons and that neurons derived from induced pluripotent stem cells from PERK haplotype B PSP patients exhibited increased vulnerability to ER stress. The study also showed that induced pluripotent stem cell-derived neurons from PERK haplotype B PSP patients had higher amounts of AT8 and PHF-1 phosphoepitopes in response to tunicamycin, suggesting that decreased PERK activity due to the PERK haplotype B variants led to increased tau pathology. However, the study did not normalize the levels of hyperphosphorylated tau to levels of total tau, which was also increased after tunicamycin treatment in PERK haplotype B PSP neurons when compared to control neurons. Therefore, it is still necessary to accurately dissect the effect of PERK coding variants on PERK activity as well as their downstream consequences in neurons as well as glia.

In contrast to reports suggesting that PERK dysregulation exacerbates tau aggregation and that alleviating PERK activation might be beneficial in tau pathology, 1 study demonstrated that pharmacological PERK activation or PERK overexpression led to a reduction in hyperphosphorylated tau levels in cultured human neurons expressing wild-type 4R tau in vitro and rescued behavioral deficits and neurodegeneration in the P301S transgenic mouse model of tauopathy in vivo (205). One explanation for this discrepancy is differential activation of downstream targets of PERK, wherein the beneficial effects of PERK activation in mitigating tau pathology might be occurring through Nrf2 rather than eIF2α phosphorylation. There is evidence supporting a beneficial role for Nrf2 in tau pathology, which involves the induction of autophagy for clearance of insoluble tau aggregates in mouse models of tauopathy (206). Nrf2 activation by PERK is independent of eIF2α phosphorylation (45) indicating that the link between PERK and tau pathology might be dependent on the pathway that is activated. Although PERK and PKR were studied to elucidate tau pathology, the relationship of tau pathology with the other 2 ISR kinases, GCN2 and HRI, has yet to be studied. While whether augmented eIF2α phosphorylation plays a direct and key role in tau pathology remains unclear, evidence suggests that the dysregulation in eIF2α phosphorylation may be an underlying pathological mechanism.

## Other Protein Aggregates

Similar to tau and Aβ aggregation, ISR kinases have also been assigned important roles in mediating α-synuclein pathology in PD (207). Tunicamycin treatment of the human neuroblastoma cell line BE2-M17D overexpressing wild-type human α-synuclein was shown to increase α-synuclein oligomers compared to untreated cells. Whether this effect occurs via the activation of PERK and induction of p-eIF2α remains to be elucidated. However, these results as well as the accumulating literature illustrating the role of ISR kinases in

mediating multiple pathways of protein aggregation suggest that p-eIF2α and/or kinases that lead to its generation might be contributing to α-synuclein oligomerization and aggregation as well.

## Neuroinflammation

Induction of inflammation in the CNS is increasingly recognized as an important component of neurodegenerative diseases, particularly during the chronic stage, as inflammatory markers have been extensively observed in the brains of diseased patients (56, 58). Several stress response pathways have been linked with neuroinflammatory processes, raising the possibility that ISR kinases might play contributory roles as mediators of inflammation. For example, in LPS-administered mice, genetic *EIF2AK2* downregulation blocked neuroinflammation as observed by normalization in the levels of increased Iba-1, a marker of microgliosis, as well as a normalization tumor necrosis factor (TNF)-α and IL-6 levels (179). Other proinflammatory cytokines were also found to be modulated by PKR. Intraperitoneal injection of C16, a pharmacological PKR inhibitor, prevented quinolinic acid-induced increase in IL-1β levels in rats (208). In another study, *EIF2AK2* knockout in 5XFAD mice attenuated Iba1 immunoreactivity in the hippocampus, whereas the levels of IL-1β, TNF-α, interferon-γ, and IL-6 returned to the levels measured in wild-type mice (181). The authors further investigated whether the effect of PKR was via neurons or microglia using mixed neuronal and microglial cocultures from wild-type and *EIF2AK2* knockout mice. Upon LPS treatment, cytokine release was attenuated in cocultures where PKR was knocked out only in the microglia when compared to wild-type. However, the effect was completely abolished only when PKR was knocked out in both cell types, suggesting PKR-mediated cytokine release involves both neurons and microglia.

Although PKR is the main ISR kinase known to mediate inflammatory responses, several studies also examined the role of PERK in cytokine expression. Particularly, genetic as well as pharmacologic inhibition of PERK decreased the expression of CCL-20 and the secretion of IL-6 and CCL-2 from astrocytes under ER stress (117).

Paradoxically, other studies demonstrated that ISR downregulation augmented neuroinflammation and that ISR activation ameliorated neuroinflammation. Specifically, knocking out *EIF2AK3*, in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), led to exacerbation of inflammation as determined by an increase in the number CD3-positive T cells and CD11b-positive macrophages/microglia in the lumbar spinal cord (209). Additionally, neuron-specific *EIF2AK3* knockout augmented axon degeneration and impaired clinical recovery in this model. The authors suggested that the potential mechanism underlying the exacerbated inflammation associated with EAE in *EIF2AK3* knockout mice was not mediated by the peripheral immune cells but rather arose via the facilitation of tissue damage in the CNS, mainly axonal degeneration. In a model of intracerebral hemorrhage which included increased p-eIF2α levels, treatment with recombinant GCN2 was associated with decreases in the levels of IL-1β and TNF-α, inhibition of neutrophil infiltration, and improvement in neurological function (210). These beneficial effects of GCN2 were abolished by blocking eIF2α, indicating that this process was dependent on eIF2α. Furthermore, in a mouse model, GCN2-deficient regulatory T cells displayed impaired migration in the presence of a CCL2 gradient, suggesting that the role of GCN2 in mediating neuroinflammation might occur via peripheral immune cells (211). No studies to date have addressed the role of HRI in neuroinflammation. Nonetheless, studies increasingly ascribe neuroprotective roles for the other ISR kinases in neurodegenerative diseases with an inflammatory component such as MS. Importantly, these proposed roles also implicate that, in addition to their functional roles in neurons, ISR kinases may also play key roles in nonneuronal cells via the modulation of inflammatory processes that are persistent in neurological disease.

# PHARMACOLOGICAL INTERVENTIONS FOR MODULATING eIF2α PHOSPHORYLATION

Given the overwhelming evidence for dysregulation of eIF2α phosphorylation as a common mechanism in several diseases, substantive efforts have been spent to identify and develop pharmacological compounds that target different components of this pathway. In this section, we review the inhibitors and activators of ISR kinases as well as other compounds that impact downstream targets (Fig. 2). The evidence thus far strongly implicates ISR signaling as an important mediator of neurodegeneration. However, whether activating or inhibiting interventions are more beneficial remains a slightly ambiguous question of stress context.

## PERK Inhibitors

All ISR kinases converge on eIF2α phosphorylation, which, as elucidated above, is considered a central hub that determines cell-fate outcomes in conditions of neurodegeneration. However, as the most extensively studied ISR kinase in efforts to mitigate neurodegenerative diseases, PERK has been the main target for the development of pharmacological inhibitors aimed at suppressing the ISR (212). GSK2606414, a specific PERK inhibitor, is a highly selective and potent compound that can effectively cross the blood-brain barrier and thus was used as the main pharmacological agent to assess the role of PERK in neurodegeneration (213). A year later, the same group reported the optimized PERK inhibitor GSK2656157 (214). The first study investigated the utility of GSK2606414 in prion-infected mice, which exhibit sustained eIF2α phosphorylation and neurodegeneration in response to misfolded prion proteins (60). In this prion model, pharmacological PERK inhibition using GSK2606414 at early disease stage rescued prion-induced neurodegeneration and reversed the behavioral abnormalities determined by the novel object recognition test and burrowing activity (94). Similarly, GSK2606414-mediated PERK inhibition prevented substantia nigra degeneration in a toxin-induced PD model in mice (215). Moreover, the PERK/eIF2α inhibition rescued the synthesis of critical synaptic proteins which allowed neuronal recovery. Furthermore, GSK2606414 led to decreased levels of

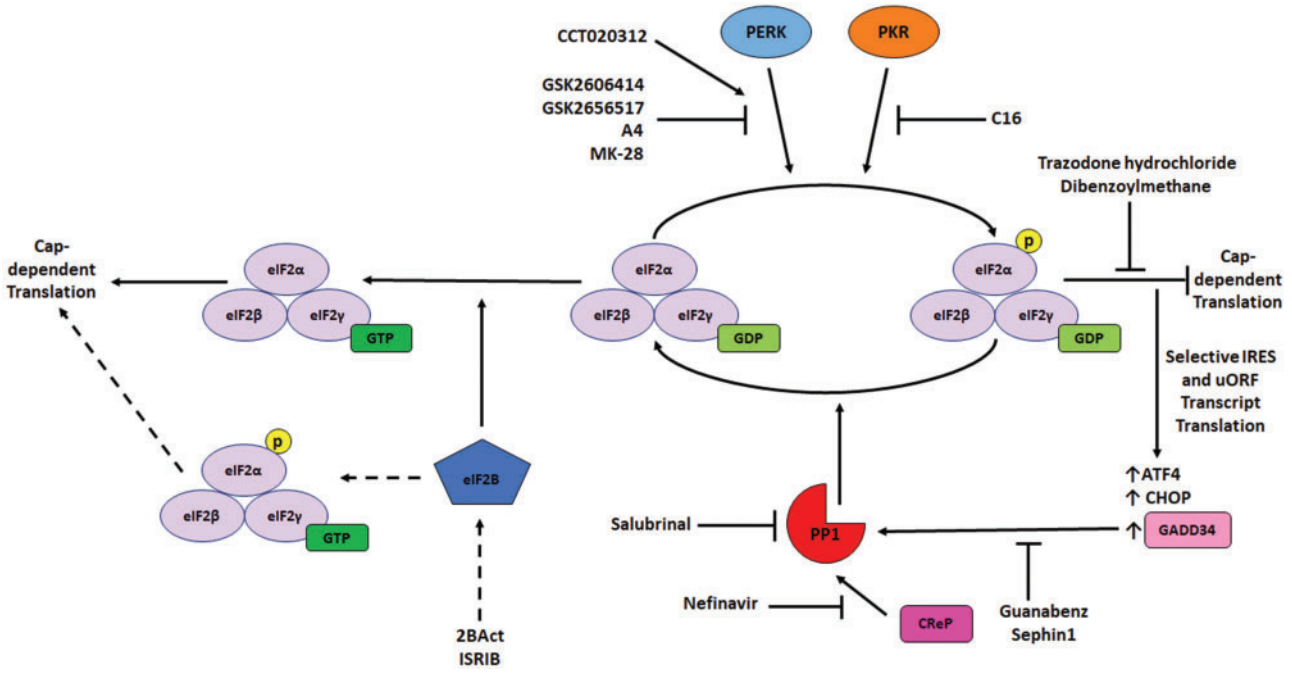


**FIGURE 2.** Pharmacological interventions targeting integrated stress response (ISR) signaling. The ISR can be pharmacologically targeted and modulated at many levels. Specific inhibitors of 2 of the branch kinases, protein kinase R (PKR), PKR-like endoplasmic reticulum (ER) kinase (PERK) have been developed to reduce eIF2α phosphorylation and prevent downstream ISR signaling. On the other hand, inhibitors of protein phosphatase 1 (PP1) and the cofactors which direct it to p-eIF2α under different conditions have been developed to raise p-eIF2α levels and prolong ISR signaling. On a more encompassing level, activators of eIF2B have been developed to allow it to interact with eIF2 even when phosphorylated, thus removing the translational block from ISR signaling. Additional repurposed drugs have also been shown to alleviate the translational block cause by eIF2 phosphorylation without dephosphorylation; however, the mechanism of this reversal is not known, whether through eIF2B or otherwise.

hyperphosphorylated tau and neurotoxicity in the rTg4510 mouse model of tauopathy, underlining the significance of PERK in mediating downstream protein aggregation (202). GSK2606414 has also been shown to reverse RAN translation in sodium arsenite- and MG132-treated cells, as well as in cells exposed to an excitotoxic glutamate insult (157, 159). In another study, intracerebroventricular injection of GSK2646157, the optimized inhibitor, reduced inflammatory and apoptotic factors associated with early brain injury after acute subarachnoid hemorrhage model in rats, as well as improved prognosis (216). This study did not show improvements in mortality or neurological deficits, however, perhaps because of the acute nature of the stress. Therefore, PERK inhibition can rescue neurodegenerative phenotypes in various disease models and does not appear specific to 1 type of neuropathological hallmark or neurodegenerative disease, suggesting that controlling PERK activity might be beneficial for a wide range of diseases.

However, in all the aforementioned studies, mice treated with GSK2606414 exhibited mild hyperglycemia and reduced body weight. Given the major role of PERK as a regulator of glucose homeostasis in the pancreas, effective systemic PERK inhibition might be associated with significant blood glucose dysregulation. Although GSK2606414 was reported to be specific for PERK and was shown to be effective in ameliorating neurodegeneration in several disease models, the associated alterations in glucose metabolism precludes its evaluation in humans and highlights the complexities of identifying CNS-PERK inhibitors without peripheral adverse effects. Intriguingly, MK-28, a derivative of an older PERK inhibitor molecule, A4, which was developed based on structural determinants was recently shown to have low pancreatic cell toxicity in mouse pancreatic cells and whole animals, to have no effect on blood glucose in wild-type mice, and to decrease elevated blood glucose found in the R2/6 transgenic mouse model of HD (217, 218). The study also demonstrated that MK-28 reduced cytotoxicity in a cellular HD model, and improved cognitive and motor behaviors as well as survival in a mouse model (218).

## PKR Inhibitors

In a screen aimed to identify specific inhibitors of PKR, from 26 ATP-binding site-directed inhibitors an oxindole/imidazole compound termed C16 was confirmed to inhibit PKR autophosphorylation, which is necessary for its activation; furthermore, C16 treatment rescued the PKR-induced translational block in rabbit reticulocytes (219). C16 has been shown to be effective in inhibiting PKR-induced toxicity in the SH-SY5Y neuronal cell line, both when mediated by treatment with tunicamycin or β-amyloid peptide (126, 220). The beneficial effects in response to a traditional ER stressor,

tunicamycin, as compared to how PERK inhibitors sensitize cells to ER-stress-mediated death, highlight PKR's more traditional apoptotic roles. Also, in contrast to PERK inhibitors, acute systematic administration of C16 in rats encouragingly inhibited apoptotic PKR signaling without simulating proliferative mTOR signaling (221). As mentioned in one of the previously discussed studies, Couturier et al (119) showed that C16 reduced levels of caspase 3 and inflammatory cytokines such as TNF-α, IL-1α, IL-1β, and IL-6 in peripheral blood mononuclear cells with activated PKR, isolated from AD patients. Also mentioned previously, the study by Tronel et al (208) showed that PKR inhibition using C16 attenuated neuroinflammation in an excitotoxic neuroinflammatory model where rats were treated with quinolinic acid. In that study, the authors demonstrated that C16, administered intraperitoneally 24 and 2 hours before as well as 24 hours after quinolinic acid injection, crossed the blood-brain barrier and decreased quinolinic acid-mediated neuronal loss. There were no reported deleterious side effects of this C16 treatment, although this was not a chronic treatment paradigm, and it remains possible that longer treatment periods with PKR inhibitors might recapitulate the adverse effects observed PERK inhibitors. Therefore, it remains uncertain whether long-term systemic treatment with ISR kinase inhibitors such as C16 might have systemic adverse effects.

## Salubrinal

Salubrinal is a small molecule identified during a screen for compounds that protected cells from ER stress-mediated apoptosis (222). Salubrinal inhibits eIF2α dephosphorylation by PP1, regardless of whether it is mediated by either of its specificity cofactors: the constitutively expressed CReP or by ER stress-induced GADD34. This inhibition is sufficient to induce elevated eIF2α phosphorylation and upregulation of downstream genes even in the absence of stress (222).

Several studies to date demonstrated the efficacy of salubrinal in ER stress and neurodegeneration: For example, in a study using SH-S5Y5 neuronal cells treated with rotenone, which induces ER stress and reduces the levels of parkin protein, to recapitulate PD, salubrinal treatment led to an increase in ATF4 expression and a reduction in cell death (223). Salubrinal also attenuated disease in a mutant α-synuclein transgenic mouse model of PD; however, in that model, the disease onset was correlated with the induction of an atypical UPR that resulted in chaperone upregulation in the absence of eIF2α phosphorylation (75). In models of ALS, specifically *Caenorhabditis elegans* and zebrafish expressing mutant *TARDBP*, encoding mutant *TDP-43*, salubrinal treatment caused reduced oxidative stress, neurodegeneration, and paralysis (224). Additionally, in cultured rat neurons and mouse microglia treated with Aβ peptide to model AD, salubrinal attenuated both neuronal death and microglial activation (225). Of note, the benefits of salubrinal were attributed not to the alleviation of ER stress but inhibition of both IKK and subsequent NF-kB activation. In a study investigating mild traumatic brain injury (TBI) in a mouse model, salubrinal rescued memory deficits as well as neuronal integrity after injury; however, the mice also exhibited reduced eIF2α phosphorylation after injury (226). Another study using blast-induced injury in rats demonstrated that salubrinal was neuroprotective in TBI by reducing markers of stress and ameliorating behavioral deficits (227).

Of note, most of these studies are showing salubrinal treatment to be beneficial in models which activate an atypical ISR, or in cells, using acute treatments to model disease. In these instances, eIF2α phosphorylation levels can be low, and perhaps even suboptimal for an adaptive stress response. Traditionally, unresolved ER stress signaling has been proposed as a mechanistic link between acute neurotrauma and progressive neurodegenerative disease. Thus, these data strongly suggest that salubrinal might be beneficial in acute but not chronic neurodegenerative conditions in which increased eIF2α phosphorylation is observed, as may be expected from the initial adaptive versus prolonged maladaptive phases of the ISR. Indeed, salubrinal treatment of prion-infected mice 8 weeks postinfection led to exacerbated disease progression, including increased neurotoxicity and decreased survival (94). Intriguingly, the authors noted the absence of GADD34 upregulation during disease progression despite chronic ER stress, which might at least partially explain the neurodegenerative phenotype that was enhanced by salubrinal.

A related molecule, sephin1, which inhibits the PP1 cofactor GADD34, was shown to prolong disease onset in mice with EAE, modeling MS, and correlated with increased eIF2α phosphorylation and myelination (228). However, eIF2α was only increased at earlier timepoints that were assessed; at late-stage disease, sephin1 led to a significant increase in inflammation. Another GADD34 inhibitor, guanabenz, was demonstrated to have both beneficial and detrimental roles in different models of ALS (224, 229, 230) and was shown to rescue neuronal toxicity in models of SCA and PD (231, 232). Additionally, nelfinavir, an antiretroviral drug that inhibits the HIV protease, inhibited constitutive eIF2α dephosphorylation via downregulation of the PP1 cofactor CReP (233). This aligns with the theory that persistence of HAND in patients on ART may be partially due to neurotoxicity of the ART drugs themselves over time and with aging (234, 235).

In primary neurons, basal eIF2α phosphorylation was found to increase the antioxidant capacity downstream of ATF4 induction in neurons and, in following, salubrinal treatment led to resistance to oxidative glutamate toxicity (236). However, persistently high levels of p-eIF2α are evidently toxic. Studies utilizing salubrinal capture the complexity of this dichotomy and illustrate that while the outcome might be dependent on the type and duration of stress, there remains ambiguity concerning the determinants of the switch between adaptive and maladaptive outcomes.

## PERK Activator

CCT020312, also known as PERK activator (PA), was discovered in a cancer study screen to identify small molecules that activate the G1/S cell cycle checkpoint (237). In that study, PA was determined to act through PERK-mediated p-eIF2α, with the pursuant translational block triggering cell cycle arrest in G1. Intriguingly the observed PERK activation happened independent of ER stress and without activation of

the other UPR branches, demonstrating its PERK selectivity (237). In that context, induction of apoptosis by PERK activation was beneficial, facilitating proliferation control and sensitizing cells to chemotherapy-associated stress. This initial study falls in line with the overall hypothesis that extensive eIF2α phosphorylation and PERK activation, particularly in the absence of activation of the other arms of the UPR, are cytotoxic. However, in another study, mentioned above, PA was used to mitigate tau pathology both in vitro and in vitro (205). Of note, this study also reported downregulation of both total and phosphorylated eIF2α protein levels in the cortex of PSP patients, in comparison to healthy controls, while both PERK and NRF2 were upregulated in both their total and phosphorylated forms (205). As discussed above, the benefit of PERK activation in this case seems to stem from upregulation of adaptive ISR signals distinct from eIF2α phosphorylation.

Even with these caveats, successful studies utilizing salubrinal and PA to ameliorate neurodegenerative phenotypes demonstrate that activation of the ISR can be beneficial in the right context. Going forward, targeting/upregulating the adaptive ISR is of therapeutic interest. PA appears to achieve this goal, but the mechanism is unknown, not to mention unvalidated in the context of elevated eIF2α phosphorylation. The small molecule discussed next, integrated stress response inhibitor (ISRIB), is another potential example. Thus far lacking nonsmall molecule modalities, such as CRISPR-mediated adaptive upregulation, may lead to more fruitful therapeutic interventions enhancing adaptive signaling rather than inhibiting maladaptive.

## Integrated Stress Response Inhibitor

Pathological implications of the substantial overlaps among the ISR kinase pathways in neurodegenerative conditions are the direct effects of eIF2α phosphorylation. A small molecule, ISRIB, which was discovered in a cell-based screen for PERK inhibitors, was found to increase cellular resistance to eIF2α phosphorylation without a decrease in PERK phosphorylation (238). Sidrauski et al also determined the *trans*-ISRIB isoform to be about more than a 100-fold more effective than *cis*-ISRIB, and as such the *trans* isoform has been used since. ISRIB interacts with eIF2B, allowing GEF activity even in the presence of p-eIF2α (239). Specifically, cryoelectron microscopy showed that ISRIB bound the β and δ subunits of the core regulatory complex, stabilizing a rate-limiting intermediate in the assembly of the decamer, which is the active form (240, 241). The binding of ISRIB to eIF2B not only prevents but also reverses the formation of stress granules, which are a common feature of neurodegenerative conditions, following ER stress (242). Additionally, ISRIB was shown to reverse RAN translation in sodium arsenite- and MG132-treated cells (159). Cells treated with ISRIB were sensitized to PERK-driven ER stress; however, ISRIB-treated mice exhibited enhanced spatial and fear-associated learning, in line with the role of translation in memory consolidation (238). Thus, ISRIB highlights both the beneficial and malignant aspects of eIF2α signaling and demonstrates the ability to target the latter.

Indeed, follow-up studies using ISRIB demonstrated its beneficial effects in various models of neurodegeneration. Specifically, in prion-infected mice ISRIB treatment partially restored translation rates and reduced ATF4 levels, resulting in neuroprotection and increased survival without toxicity to ER stress-prone pancreatic cells (243). The same study also demonstrated that ISRIB was able to cross the blood-brain barrier, illustrating its potential as a therapeutic agent. Another study, investigating TBI modeled in mice, showed that ISRIB reversed cognitive deficits, even when administrated weeks after injury, and that these effects persisted even after the termination of treatment (244). Among studies investigating the maladaptive outcomes of eIF2α phosphorylation, Briggs et al (245) found that ISRIB treatment of the PS19 mice expressing the mutant P301S tau led to the augmentation of the tau pathology, as evidenced by increased AT8 levels and phosphorylated tau-positive inclusions, albeit a modest restoration of spatial acquisition deficits reported in other studies. Additionally, in cells overexpressing APP as a model of AD, ISRIB was able to ameliorate cell death in a manner independent of Aβ production (246). Overall, these data suggest that ISRIB might be beneficial by rescuing the translational block induced by p-eIF2α rather than by a direct effect on amyloidogenesis or tau pathology. Of note, ISRIB was also shown to alleviate the neuronal toxicity of elvitegravir, an antiretroviral drug proposed to play a role in the pathogenesis of HAND, providing additional neurodegenerative conditions where it might have utility as an adjunctive therapy (247).

Intriguingly, a recent study examining the effects of ISRIB during viral infection found that ISRIB was able to restore translation only during early infection when eIF2α phosphorylation levels were low, and that its effect was overcome above a critical threshold, independent of the trigger (248). Critically, the authors suggested that infection represented an acute stress condition where the beneficial outcomes of ISR activation are predominant, in comparison to neurodegeneration, in which chronic low-level stress facilitates predominance of the maladaptive ISR. Thus, the suggested eIF2α phosphorylation threshold may act to limit ISRIB activity to a window of chronic stress that could account for its lack of overt toxicity in vivo. In following, 2BAct, a recently developed derivative of ISRIB with increased solubility and pharmacokinetics, was also shown to restore translation under chronic ER stress conditions and sustained eIF2α phosphorylation (249). In a mouse model of vanishing white matter disease, which arises from mutations in *EIF2B1*, encoding mutant that diminish its activity, treatment with 2BAct or ISRIB prevented myelin loss, reactive gliosis, and motor deficits (250). These accumulating data not only emphasize the central role of eIF2α phosphorylation in a myriad of neurodegenerative conditions affecting distinct cells in the CNS but also raise hope for treatment strategies that can be utilized to alleviate or resolve damage in these diseases with complex pathological mechanisms.

## Repurposed Compounds Targeting eIF2α Phosphorylation

Due to the pancreatic toxicity of common PERK inhibitors, Halliday et al examined the efficacy of other compounds for their inhibitory potential in the eIF2α pathway to identify

potential therapeutic compounds. In a screen of 1040 FDA-approved drugs, the authors assessed the candidates based on their ability to overcome the tunicamycin-induced development block in *C. elegans* as well as their potency in inhibiting CHOP expression. They also examined the efficacy in attenuating neurodegeneration and neurological deficits in prion-infected mice and the rTg4510 mouse model of tauopathy, in the absence of pancreatic toxicity (83). In that study, the authors identified 2 drugs, trazodone, an antidepressant, and dibenzoylmethane, a curcumin analog with anticancer properties, that were effective in inhibiting the PERK/eIF2α signaling. Both compounds were able to rescue neurodegeneration and increase survival in the mouse models that were tested. They were also shown to mitigate MG132, sodium arsenite, and glutamate-induced RAN translation (157). Thus, these 2 molecules have been identified as new potential therapeutic agents in mediating dysregulation of p-eIF2α in disease without the adverse side effects of previously developed compounds targeting eIF2α phosphorylation.

## Conclusions and Perspectives

While the ISR is a canonically adaptive signaling cascade, chronic ISR activation and dysregulated p-eIF2α can contribute to neurodegenerative phenotypes via canonical apoptotic induction as well as translation-dependent deficits that result in neuronal dysfunction. Given this sensitive dichotomy, and the prevalence of genetic implications of the ISR in neurodegeneration, targeting the ISR kinases and their downstream signaling components could make for fruitful pharmacogenetic studies. Furthermore, extensive compelling evidence suggests the downstream induction of, or feed-forward contribution to, amyloidogenesis, tau pathology, and neuroinflammation as major mechanistic drivers of neurodegeneration. Still, while the myriad pathways involved in eIF2α phosphorylation make compelling targets for pharmacological intervention, supported by promising studies, the homeostatic activities of these targets and signaling pathways must be considered and adjusted for when they are being manipulated, in order to better infer their implications for therapeutic development.

As the field moves forward, efforts should be made to elucidate the roles and contributions of the lesser-studied ISR kinases to neurodegeneration. In this vein, it is imperative to consider the crosstalk and/or compensatory upregulation of the ISR kinases that might exist under canonical activation or inhibition of the others. Finally, the intersection of these signals across different cell types is highly likely to play a role in disease pathogenesis and warrants extensive further attention.

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