Trends in Molecular Medicine

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Review

Neuronal Regulation of eIF2α Function in Health and Neurological Disorders

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A key site of translation control is the phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α), which reduces the rate of GDP to GTP exchange by eIF2B, leading to altered translation. The extent of eIF2α phosphorylation within neurons can alter synaptic plasticity. Phosphorylation of eIF2α is triggered by four stress-responsive kinases, and as such eIF2α is often phosphorylated during neurological perturbations or disease. Moreover, in some cases decreasing eIF2α phosphorylation mitigates neurodegeneration, suggesting that this could be a therapeutic target. Mutations in the γ subunit of eIF2, the guanine exchange factor eIF2B, an eIF2α phosphatase, or in two eIF2α kinases can cause disease in humans, demonstrating the importance of proper regulation of eIF2α phosphorylation for health.

Highlights

Phosphorylation of eIF2α is a key regulatory target for translation control that is important in regulating translation during normal and stress conditions.

Emerging data highlight that eIF2α phosphorylation is crucial in neuronal function and impacts synaptic plasticity as well as being inappropriately increased in numerous neurodegenerative diseases.

Mutations in components of the eIF2α phosphorylation circuit give rise to human diseases, often including neurological and/or neurodegenerative pathologies.

In model systems of neurological disease with perturbed eIF2α function, therapeutic restoration of proper eIF2α control can decrease the severity of disease via targeting of eIF2α kinases or phosphatases, or by mitigating phospho-eIF2α activity.

The regulation of eIF2α phosphorylation is a promising therapeutic target for the treatment of neurological diseases.

EIF2α Is a Major Nexus of Translation Regulation in Neurological Health and Disease

The regulation of translation is an important aspect of the control of eukaryotic gene expression, and most commonly occurs during the initiation phase of translation. Initiation of translation is a multistep process wherein the mRNA–protein complex (mRNP) recruits a multifactor complex (MFC) containing the initiation factors eIF1, eIF3, and eIF5, as well as the eIF2 complex bound to GTP and the initiator tRNA, facilitating delivery of the ternary complex to the 40S ribosomal subunit [1] (Figure 1). The MFC is most commonly recruited to the mRNA by the eIF4F complex which recognizes and binds to the 5′ cap structure and positions the MFC to scan from the 5′ end to the AUG initiation codon. Once the AUG is recognized, hydrolysis of GTP by eIF2 commits the 40S ribosome to translation initiation and leads to the recruitment of the 60S subunit and entry into the elongation phase of translation initiation. After release of eIF2–GDP from the ribosome, GDP is exchanged for GTP by the guanine nucleotide exchange factor (GEF) eIF2B, preparing the eIF2 complex for another round of initiation.

The exchange of GDP for GTP on the heterotrimeric eIF2 complex by the eIF2B complex has emerged as a major node of translation control (Figure 2). In humans, four distinct kinases can be activated by various intracellular cues to phosphorylate the eIF2α subunit of the eIF2 complex on serine 51 (in humans) [2,3]. Generally, PERK (protein kinase R-like endoplasmic reticulum kinase) phosphorylates eIF2α in response to unfolded proteins in the endoplasmic reticulum (ER) as part of the **unfolded protein response** (UPR) (see Glossary), while the eIF2α kinases protein kinase R (PKR), heme-regulated inhibitor (HRI), and general control nonderepressible 2 (GCN2) respond to double-stranded (ds)RNA, oxidative stress, and nutrient deprivation and UV, respectively. However, activation of these kinases is not always restricted to specific stimuli. For example, in addition to UV and nutrient deprivation stress, the UPR can activate GCN2 [4]. The diversity of inputs that trigger eIF2α kinase activation in general and in neurons has not been fully elaborated.

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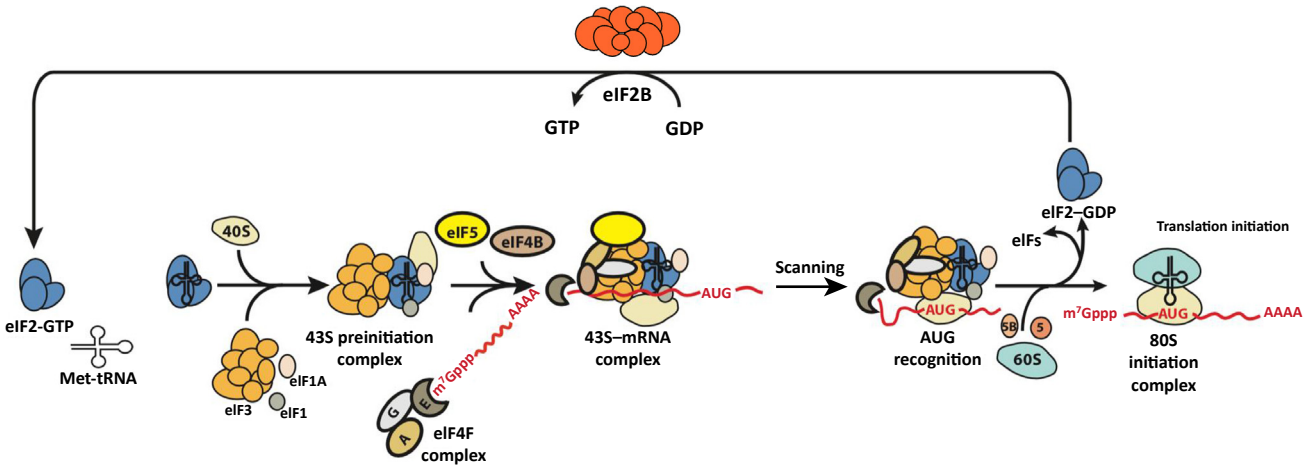

Figure 1. The Translation Initiation Pathway in Eukaryotes Comprises Several Key Steps. First, the eukaryotic initiation factor (eIF) 2B complex reloads the eIF2 complex with GTP to enable ternary complex formation. Next, the 43S preinitiation complex assembles and recruits mRNA to be translated. Scanning commences to then enable start codon recognition, and translation initiates upon formation of the 80S complex.

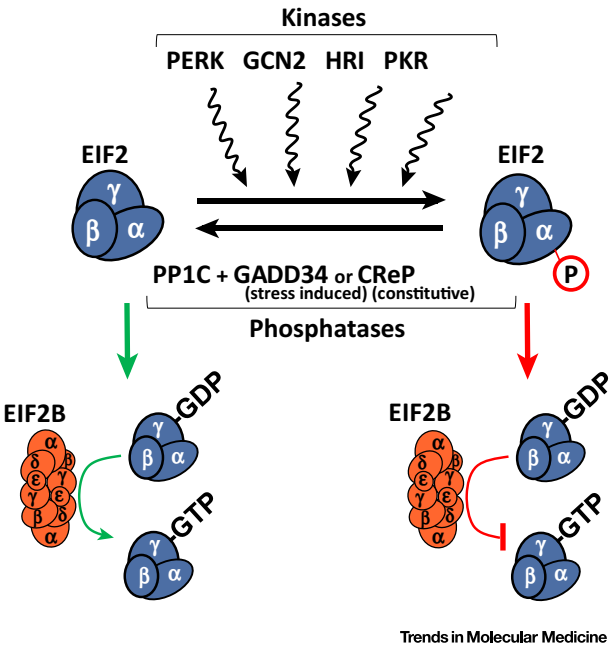

Figure 2. The Eukaryotic Initiation Factor (eIF) 2 Complex Is a Crucial Regulatory Nexus for Translation Initiation. The α subunit of the eIF2 complex is phosphorylated (P) by any of four kinases [protein kinase R-like endoplasmic reticulum kinase (PERK), general control nonderepressible 2 (GCN2), heme-regulated inhibitor (HRI) and protein kinase R (PKR)] in response to stress, and is dephosphorylated by stress-induced (GADD34, growth arrest and DNA damage inducible protein 34) or constitutively expressed (CReP, constitutive reverter of eIF2α phosphorylation) phosphatases. Phospho-eIF2α inhibits the eIF2B complex, reducing guanine exchange factor activity and limiting translation initiation.

Glossary

Amyloid β (Aβ) aggregates: formed upon cleavage of the amyloid precursor protein, amyloid β peptides are highly prone to aggregation. Extracellular Aβ aggregates are implicated in Alzheimer's disease pathogenesis.
Integrated stress response (ISR): a conserved response to extrinsic and intrinsic cellular stresses that causes global suppression of translational initiation through reversible phosphorylation of eIF2α and selective expression of stress-induced genes at the levels of transcription and translation.
MEHMO syndrome: a rare syndrome defined by mental retardation, epileptic seizures, hypogenitalism, microcephaly, and obesity caused by mutations in the *EIF2S3* gene, which encodes the gamma subunit of the eIF2 complex.
RAN (Repeat-associated non-AUG) translation: translation of repeat expansion-containing RNAs in multiple open reading frames through non-canonical (non-AUG) translation initiation, leading to generation of mono-, di-, tetra-, and penta-peptides.
Repeat expansion diseases: a class of neurological disorders including Huntington disease, myotonic dystrophy, and fragile X

Phosphorylation of eIF2α increases its affinity for the GEF eIF2B, and thereby limits the exchange of GDP for GTP [5]. Of note, under conditions of increased tRNA and eIF2γβ levels, the α subunit of the eIF2 heterotrimer is unnecessary for ternary complex formation and translation in yeast [6]. Phosphorylation of eIF2α reduces the concentration of eIF2–GTP complexes, and thereby decreases bulk translation. However, mRNAs that contain upstream open reading frames (uORFs) can actually exhibit increased translation from the major ORF because the kinetics of eIF2–GTP and eIF3 reassociation with scanning ribosomes are slow

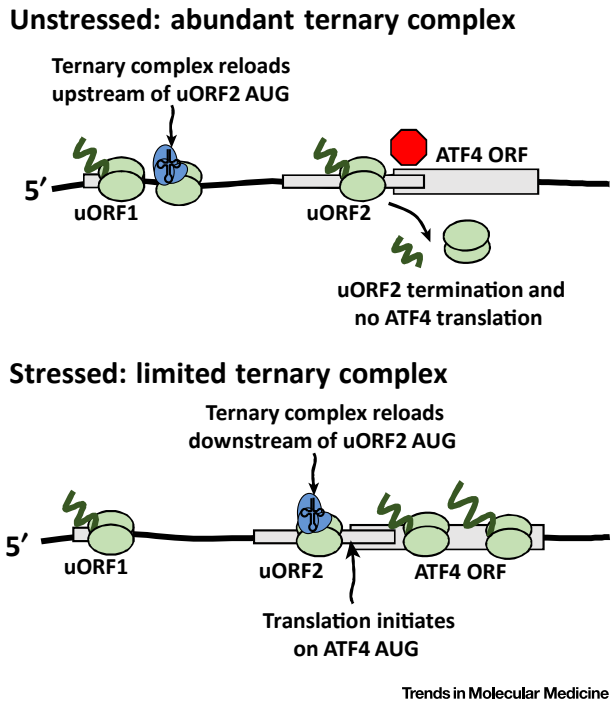

Figure 3. Translation Initiation of Mammalian Activating Transcription Factor 4 (ATF4) mRNA Harboring Inhibitory Upstream Open Reading Frames (uORFs) Is Enhanced During Stress when the Ternary Complex is Limited. Adapted, with permission, from [22].

enough to bypass the upstream inhibitory uORFs [7] (Figure 3). Similarly, mRNAs that utilize non-canonical translation initiation mechanisms such as internal ribosome entry sites (IRESs) can show increased translation with eIF2α phosphorylation [8,9]. Further, different stresses can cause similar and distinct changes in the functional transcriptome (corresponding to the activation of specific eIF2α kinases), as has been shown in studies in mouse liver cells [10,11], likely as a result of either eIF2α-independent stress-activated gene regulation mechanisms or cell type-specific differences in the relative abundances of each kinase. Thus, eIF2α phosphorylation is important in the downregulation of bulk translation under a wide variety of conditions, but is equally (or even more) important in allowing the enhanced translation of specific mRNAs.

The phosphorylation of eIF2α in response to a wide variety of stresses is a key part of the overall response to stress, which is referred to as the **integrated stress response** (ISR) [2,12]. The ISR involves initial activation of an eIF2α kinase (e.g., PERK), leading to eIF2α phosphorylation, and a decrease in bulk translation activity with enhanced translation of some specific mRNAs. The enhanced translation of specific mRNAs, such as those encoding transcription factors ATF4 and CHOP, leads to transcriptional induction of downstream genes which modulate the recovery from stress, or, if the stress is too extreme, can trigger apoptosis (reviewed in [13]).

Evidence now shows that the control of eIF2α phosphorylation plays important roles in neurons, both in regulating synaptic plasticity and in response to neurodegenerative diseases. This regulation is complex because all four eIF2α kinases are expressed in the brain, although HRI expression is very low (reviewed in [14]). Of note, differential abundances of each kinase in

mental retardation syndrome caused by repeat expansion mutations that lead to pathogenic loss or gain of function.

Tau oligomers: the neuronal microtubule-associated protein tau can aggregate and form intracellular tau oligomers and neurofibrillary tangles, histological hallmarks implicated in the pathogenesis of numerous tauopathies including Alzheimer's disease and frontotemporal dementia.

Unfolded protein response (UPR): a coordinated cellular response to endoplasmic reticulum (ER) stress that activates ATF6 (activating transcription factor 6), PERK (protein kinase R-like endoplasmic reticulum kinase), and IRE1 (inositol-requiring enzyme 1) to reprogram transcriptional and post-transcriptional gene regulation, ultimately to reduce cellular protein production and enhance protein folding capacity in the ER.

Vanishing White Matter Disease (VWMD): a rare fatal leukodystrophy caused by mutations in genes encoding any subunit of the EIF2B complex. Episodic, progressive deterioration of the white matter can occur following trauma or illness.

Wolcott–Rallison syndrome: a rare syndrome due to mutations in PERK (protein kinase R-like endoplasmic reticulum kinase) that causes microcephaly, intellectual disability, developmental delays, non-autoimmune diabetes, liver and renal dysfunction, and rarely neurodegeneration in childhood.

different brain regions could confer specific functional outcomes upon stress in disease contexts. Moreover, mutations that alter this regulatory circuit can have dramatic deleterious consequences that cause disease. We review below the contributions of eIF2α phosphorylation to neuronal health in both normal and disease contexts.

eIF2α Phosphorylation Modulates Synaptic Plasticity

Numerous observations provide genetic data that eIF2α phosphorylation affects synaptic plasticity (reviewed in depth in [14,15]). For example, mice heterozygous for the S51A mutation, which prevents eIF2α phosphorylation by changing the phosphorylation site, have enhanced long-term potentiation (LTP) and memory (LTM) [16]. Similarly, an increase in PKR activity in hippocampal neurons impaired long-lasting (L)-LTP and LTM in mice [17], while hippocampal infusion of sal003, a small compound that inhibits dephosphorylation of phospho (p)-eIF2α, limited L-LTP and LTM [16]. Moreover, consistent with PKR activity limiting memory formation, inhibition or depletion of PKR enhances learning and memory in mice [18].

The role of eIF2α phosphorylation in memory formation is complex, and alterations in eIF2α phosphorylation can have different effects depending on the experimental context. For example, mice lacking the GCN2 kinase show a lower threshold for L-LTP with weak training regimens, but are deficient at memory consolidation with stronger training paradigms [19]. The dual effects of alterations in eIF2α phosphorylation on learning and memory highlights that this is a crucial regulatory site and needs to be able to be modulated in multiple ways for the appropriate outcome.

The mechanism by which eIF2α phosphorylation affects L-LTP and LTM is likely to involve altering the translation of specific neuronal mRNAs that contain uORFs, and therefore will have enhanced translation when eIF2α is phosphorylated. For example, the activating transcription factor 4 (ATF4) mRNA has two uORFs, and the ATF4 mRNA shows increased translation at the second downstream uORF when eIF2α is phosphorylated [20–22] (Figure 3). *ATF4* encodes a transcription factor that can induce downstream genes, and also binds to and inhibits CREB, a key transcriptional factor for activating mRNAs required for synaptic plasticity [23]. Consistent with ATF4 being an important target in this control circuit, eIF2α phosphorylation generally correlates with increased ATF4 expression and impaired L-LTP and LTM, leading to defects in memory and learning [17,19,24]. Correspondingly, inhibition of eIF2α dephosphorylation with Sal003 in hippocampal slices from ATF4$^{-/-}$ mice had no effect on L-LTP (e.g., [16]). Thus, regulation of ATF4 translation by eIF2α phosphorylation is likely to play an important role in the modulation of L-LTP and LTM.

Notably, recent work demonstrated that translation initiation on the second ATF4 uORF is blocked under normal conditions by m^{6}A methylation because depletion of the demethylase ALKBH5 (alkB homolog 5, RNA demethylase) suppressed ATF4 production, while depletion of the methyltransferase METTL3 (methyltransferase-like 3) caused increased *ATF4* induction during amino acid deprivation stress [25]. This implies that ATF4 regulation and, as such, the effects of eIF2 phosphorylation on synaptic plasticity, may be affected by m^{6}A modification of mRNAs.

One anticipates that altered translation of multiple other neuronal mRNAs in response to eIF2α phosphorylation will also affect synaptic modulation. Several other neuronal mRNAs have uORFs similar to ATF4, and those mRNAs will likely have increased translation rates upon eIF2α phosphorylation ([15] for review). For example, GADD34 mRNA has two uORFs and its translation during eIF2α phosphorylation would initiate a negative feedback loop leading to

dephosphorylation of eIF2α [26,27]. Similarly, the mRNA for protein kinase M(ζ), which is required for memory formation, has seven uORFs and has been proposed to exhibit decreased translation when PERK is activated [28]. Therefore, the post-transcriptional regulation of mRNAs at the level of translation is one mechanism by which neuronal cell function is modulated.

Pharmacological Modulation of eIF2α Phosphorylation

The effect of eIF2α phosphorylation on neuronal function identifies this circuit as a potential target for pharmacological intervention. Three classes of drugs have been identified that affect various aspects of this control process (Figure 4). Several inhibitors of the eIF2 kinases have been identified (reviewed in [29]). For example, the PERK inhibitor I, GSK2606414 [30], has been used in many experiments to manipulate eIF2α phosphorylation in animals [31,32]. Similarly, various PKR inhibitors have also been used in neuronal contexts (e.g., [33,34]). Screens have been performed to identify inhibitors of HRI and GCN2 (reviewed in [29]), and the GCN2 compounds appear to be effective in tissue culture models [35]. In addition, compounds that inhibit GADD34/PP1c or CReP/PP1c phosphatase action on eIF2 have been identified. For example, the Sal003 compound, which is an analog of salubrinal, is a cell-permeable compound that inhibits PP1c-mediated dephosphorylation of eIF2α [36]. Finally, a novel compound, ISRIB, has been identified that suppresses the negative effects of eIF2 phosphorylation [37]. While these compounds are useful research tools, the continued identification and refinement of these and other pharmacological tools to manipulate eIF2α phosphorylation in neurons will be an important area for future drug discovery. Special emphasis should be placed on evaluating the degree to which the ISR is perturbed by therapeutic modulation because complete and/or chronic inhibition or activation of components of the ISR could itself promote disease.

eIF2α Phosphorylation Occurs in Many Brain Perturbations and Degenerative Diseases

A striking observation is that eIF2α phosphorylation, and/or activation of aspects of the ISR, occur in a wide variety of brain perturbations and neurological diseases. For example, eIF2α phosphorylation, stress-induced gene expression, and/or activated eIF2α kinases are seen in patient tissues and models of traumatic brain injury [37–39], Alzheimer's disease [40–42], amyotrophic lateral sclerosis [31,43], **repeat expansion diseases** [44–48], Parkinson's disease [49], and a wide variety of leukodystrophies (reviewed in [50,51]; including Charcot–Marie–Tooth disease [52] and Pelizaeus–Merzbacher disease [53,54]). Thus, activation of the ISR and phosphorylation of eIF2α is a common factor in many neurodegenerative diseases.

Different mechanisms contribute to activating kinases of eIF2α in these disease conditions (Table 1). For example, in several hypomyelination diseases, point mutations affecting the folding of very highly expressed proteins that traverse through the ER lead to the accumulation of unfolded proteins in the ER and trigger an unfolded protein response and PERK activation [52–56]. Similarly, **amyloidβ(Aβ) aggregates** and **tau oligomers** in Alzheimer's disease models can trigger the UPR through their association with the ER to activate PERK, GCN2, or PKR [28,57–60], and prion-like TDP-43 aggregates can also induce eIF2α phosphorylation [31,46]. Therefore, the aberrant accumulation of misfolded or aggregation-prone proteins can contribute to ISR activation in many neurological disorders.

Neuroinflammation, which is widely observed in many neurodegenerative disorders, is also thought to contribute to activation of the ISR and, conversely, can also result from chronic ISR activation (reviewed in [61]) because cytokines and NF-κB are upregulated during the ISR

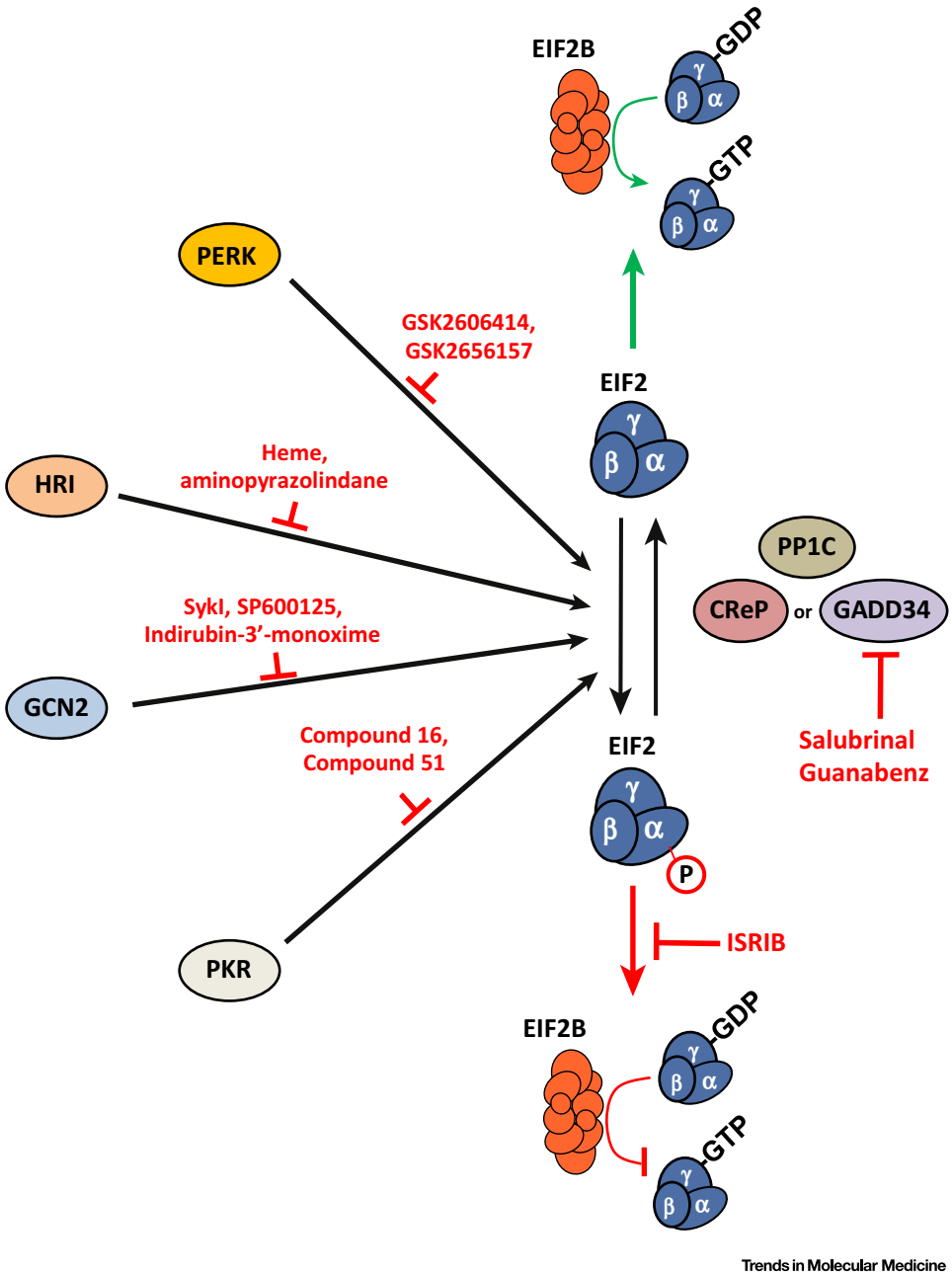

Figure 4. Small-Molecule Modulators of Phospho (p)-Eukaryotic Initiation Factor (eIF) 2α Activity. These include Inhibitors of the eIF2α kinases PERK (protein kinase R-like endoplasmic reticulum kinase) [30,130], HRI (heme-regulated inhibitor) [131–133], GCN2 (general control nonderepressible 2) [35], PKR (protein kinase R) [134], p-eIF2α phosphatase GADD34 (growth arrest and DNA damage-inducible protein 34) [36,135], and an inhibitor of p-eIF2α activity that increases eIF2B function [37].

[3,62,63]. In models of Alzheimer's disease, the proinflammatory cytokine TNF-α is required for activation of PKR and subsequent phosphorylation of eIF2α triggered by Aβ oligomers [41]. Similarly, demyelinating lesions in patients and murine models of multiple sclerosis exhibit elevated markers of ER stress [64–66], and interferon (IFN)-γ causes ER stress in oligodendrocytes [67–69]. This may occur via rapid upregulation of protein biosynthesis that burdens

Table 1. Chronic Activation of Stress-Response Pathways Resulting in eIF2α Phosphorylation Is a Common Occurrence in Neurological Disease[a]

Disease	Potential stressor	Stress-activated kinase	Refs
Prion diseases (e.g., infectious prion disease, Creutzfeldt–Jakob disease)	RNA–protein aggregates, protein aggregates	PKR	[76–80]
Repeat-expansion diseases (e.g., amyotrophic lateral sclerosis/ frontotemporal dementia, Huntington's disease)	Protein aggregates, RNA–protein aggregates, peptides from RAN translation, dsRNA-like structures	PERK, PKR	[31,46,48,74,75]
Alzheimer's disease (familial or associated with Down's syndrome)	Aβ protein aggregates, tau oligomers, TNF-α	PKR, PERK, GCN2	[28,41,57–60]
Multiple sclerosis and inflammatory leukodystrophy models	IFN-γ-mediated translation induction?	PERK, PKR	[67–73]
Heritable hypomyelination disorders (e.g., Charcot–Marie–Tooth disease, Pelizaeus–Merzbacher disease)	Misfolded, highly abundant oligodendrocyte/Schwann cell proteins retained in the ER	PERK	[51,53–56,82]
Parkinson's disease, Lewy body dementia	Defective mitochondria, protein aggregates	PERK	[49,94,136]

[a]Abbreviations: PKR, protein kinase R; PERK, protein kinase R-like ER kinase; GCN2, general control nonderepressible 2; TNF-α, tumor necrosis factor α.

the ER [70] because treatment with IFN-γ substantially alters the functional transcriptome [71]. It has also been suggested that IFN-γ mRNA can activate PKR, thereby causing phosphorylation of eIF2α and suppressing its own expression [72,73]. Neuroinflammation may be a key factor in contributing to hyperactivation of the ISR in neurodevelopmental and degenerative disorders.

A third potential mechanism for the activation of eIF2α kinases is through pathogenic or disease-associated RNAs generated in disease contexts. Brain tissue from patients with Huntington's disease, a repeat-expansion disease caused by trinucleotide repeats in the *HTT* gene, or from mouse models of the disease, have higher levels of activated PKR [48]. Because PKR could be copurified with *HTT* mRNA containing repeats, but not with wild-type *HTT* mRNA from patient-derived brain tissue lysates [48], and mutant *HTT* and other RNAs with pathogenic repeat expansions may form dsRNA-like structures [74,75], it is possible that PKR can bind to repeat expansion-containing RNAs as substrates to activate the ISR.

PKR may also be activated by some RNA–protein complexes. For example, the conversion of cellular prion protein to the pathogenic form is enhanced by RNA [76,77], and prions can form RNA–protein aggregates [78,79] that are capable of activating PKR [80]. Similarly, the presence of large stress granules, which are sizable assemblies of non-translating mRNPs, can cause PKR activation [81]. Therefore, aberrant RNAs and RNP complexes could also contribute to ISR activation through PKR signaling in various neurodegenerative conditions.

Protective Effects of eIF2α Phosphorylation in Neurological Disease Contexts

Although the phosphorylated form of eIF2α is observed in a variety of neurological disorders, phosphorylation of eIF2α could in principle be beneficial to neurons or glial cells. Murine models of hypomyelination and demyelination disorders have lent support to the idea that translational suppression during stress reduces the burden of protein and/or lipid biosynthesis in the ER of

myelin-forming oligodendrocytes, resulting in diminished mRNA translation and alleviating disease. For example, mouse models of Charcot–Marie–Tooth disease that exhibit chronic UPR activation can be partially rescued by the inactivation or depletion of the p-eIF2α phosphatase GADD34 [55,82,83], which causes an increase in the level of p-eIF2α and a concomitant reduction in protein biosynthesis. Correspondingly, treatment with salubrinal partially rescues demyelination due to IFN-γ treatment in cultured hippocampal slices [84]. Similarly, activation of an ER stress response in a mouse model of multiple sclerosis before disease onset was protective and limited disease severity [69]. Finally, depletion of the eIF2α kinase PERK exacerbates IFN-γ-induced oligodendrocyte and myelin loss [67].

Several studies have also demonstrated that p-eIF2α plays a positive role in the context of other neurological disorders which do not primarily result in dysmyelination. For instance, salubrinal treatment generally protects cells from apoptosis due to chronic ER stress [36], reduces pathogenesis in a model of traumatic brain injury [85], and reduces excitotoxic cell death in a murine model of epilepsy [86]. Therefore, elevation of p-eIF2α levels appears to be beneficial in particular disease contexts.

Detrimental Effects of eIF2α Phosphorylation in Neurological Disease Contexts

Because constitutive activation of the ISR can lead to cell death, strong and chronic eIF2α phosphorylation could contribute to cell death and disease. For example, IFN-γ inhibits remyelination in association with ISR activation in cuprizone-treated and experimental autoimmune encephalitis mouse models [68], and causes apoptosis of oligodendrocytes and hypomyelination in rat oligodendrocytes in culture [67]. In support of this concept, small-molecule inhibitors of the ISR including ISRIB, which increases the activity of eIF2B and reduces the inhibitory effect of p-eIF2α to block translational suppression in biochemical assays with purified proteins and in a variety of mammalian cell cultures [87,88], confer beneficial effects in many different disease situations. Specifically, ISRIB enhances memory consolidation and learning [37], protects against cognitive impairments due to traumatic brain injury by improving long-term potentiation in the hippocampus [39], and rescues defects in sociability and heightened anxiety in a mouse model of neuropsychiatric disorders including schizophrenia and bipolar disorder [89]. ISRIB also reduces toxicity of amyloid-β in a neuronal cell culture model of Alzheimer's disease [90] and limits neuronal loss and spongiform pathology in a murine model of infectious prion disease [91]. Therefore, directly targeting GEF activity of the eIF2B complex can ameliorate some aspects of neurological disease, although it is formally possible that ISRIB has additional off-target effects that could change the functional transcriptome.

A recently reported chemical screen yielded two small molecules, dibenzoylmethane and trazodone (an antidepressant), that repress the activity of a CHOP reporter in Chinese hamster ovary cells [92] during tunicamycin (ER) stress to levels similar to those observed with ISRIB treatment [93]. It was hypothesized that these drugs may increase ternary complex levels through a mechanism similar to that of ISRIB because global translation activity was increased upon treatment of hippocampal slices from diseased mice (described below) with trazodone or dibenzoylmethane, and levels of p-eIF2α remain unchanged in the presence of either drug. Importantly, treatment with trazodone or dibenzoylmethane significantly extended lifespan and reduced neuronal loss in a mouse model of infectious prion disease, and partially rescued pathology in a mouse model of frontotemporal dementia [93]. Therefore, increasing translation activity without altering p-eIF2α levels (perhaps by modulating ternary complex levels) may serve as an important therapeutic option for alleviating neuropathogenesis.

Depletion of eIF2α kinases can also confer neuroprotective activity in some contexts. Genetic depletion of either PERK or GCN2 enhances spatial memory and rescues LTP defects caused by Aβ in a murine Alzheimer's disease model [28]. PERK inhibitor I (GSK2606414) reduces neuronal loss in *Drosophila* models of early-onset Parkinson's disease [94], and suppresses TDP-43 toxicity in *Drosophila* and murine neuronal culture models of amyotrophic lateral sclerosis [31]. In addition, the PERK inhibitor I is neuroprotective in a murine model of frontotemporal dementia [95], and prevents clinical disease and neurodegeneration in mice infected with prions [96]. Targeting PKR also improves neurologic outcomes in several disease contexts. The PKR inhibitor C16 prevents neuronal apoptosis induced by excitotoxicity in rats [34], and genetic ablation of PKR inhibits neuroinflammation associated with Aβ accumulation [97]. Further, neurodegeneration produced by thiamine deficiency can be rescued in mice through treatment with PKR inhibitors [33]. The observation that pharmacological suppression of p-eIF2α-mediated translational repression is beneficial in many disease contexts therefore indicates that activation of the ISR can contribute to neuropathology.

An additional intriguing pathogenic outcome of eIF2α phosphorylation is the selective increase in **repeat-associated non-AUG (RAN) translation** [46,47]. **RAN translation** is a non-canonical type of translation that is observed in repeat-expansion diseases including amyotrophic lateral sclerosis, spinocerebellar ataxia type 8, fragile X tremor ataxia syndrome, and Huntington's disease (reviewed in [98,99]). RAN translation occurs on multiple different repeat expansion-containing RNAs in any frame, producing mono-, di-, tetra-, and pentapeptide repeat-expansion proteins [100–103]. These polypeptides accumulate in neurons and other tissues, and can be neurotoxic [99,103]. Because repeat expansions can cause stress-inducing protein and/or RNA aggregates (Table 1), and RAN translation is upregulated when translation is downregulated during stress, targeting the ISR may prove to be doubly important as a therapeutic mechanism for these disorders. Indeed, treatment of cell cultures with ISRIB reduced RAN translation during stress [46], while treatment with Sal003, an analog of salubrinal that inhibits GADD34 and increases p-eIF2α levels, causes increased RAN translation [47]. Therefore, modulation of the ISR generally serves as a key pharmaceutical approach in many neurodegenerative and neurodevelopmental diseases.

In sum, these observations support the idea that there is an optimal range of translation activity that needs to be maintained in stressed or unstressed conditions for proper control of the ISR and optimal cell health/viability. For instance, in myelination disorders it is thought that the stage of oligodendrocyte maturation (which dictates the level of protein and lipid production in these cells) and the degree of ER stress caused by IFN-γ play a role in determining whether an ISR is beneficial or detrimental in the context of hypomyelination disorders [67–70]. However, the beneficial effects of reducing eIF2α phosphorylation in multiple contexts indicate that this is an exciting area for the development of new therapeutics.

Mutations in Components of the eIF2α GDP–GTP Cycle Cause Human Diseases

Consistent with proper regulation of eIF2α phosphorylation being important for health, mutations in several components of this circuit give rise to human diseases (Table 2). For example, over 120 different recessive mutations in any of the five subunits of the GEF eIF2B give rise to **vanishing white matter disease** (VWMD) [104,105]. VWMD is one of the most common forms of childhood leukodystrophy, although symptoms can manifest at any age. Patients with VWMD often exhibit developmental delays, spasticity, and ataxia, with progressive white matter loss and ovarian dysgenesis. The severity of VWMD is inversely correlated with age of symptom onset, and progressive white matter loss is often observed following physiological stresses

Table 2. Several Neurological and Neurodevelopmental Disorders Are Caused by Mutations in Key Regulators of the Integrated Stress Response[a]

Syndrome/neurological disease	Heritability	Zygosity	Affected gene	Mutations	Refs
Global developmental delay, microcephaly, intellectual disability, hypomyelination	Autosomal recessive	Homozygous	*PPP1R15B* (also known as *CREP*)	R658C	[127,128]
MEHMO syndrome: severe X-linked intellectual disability with hypomyelination, spasticity, and epilepsy	X-linked recessive	Hemizygous	*EIF2S3* (also known as *EIF2G*)	I222T, I259M, S108R, V151L, I465SfsTer4	[115–117,136]
Vanishing white matter disease: encephalopathy, hypomyelination, spasticity, ataxia, seizures, progressive white matter loss (especially after trauma or illness) at any age	Autosomal recessive	Homozygous or compound heterozygous	*EIF2B1*, *EIF2B2*, *EIF2B3*, *EIF2B4*, *EIF2B5*	*EIF2B5* R113H (most common) >120 identified (see [105])	[104–106,137–139]
Wolcott–Rallison syndrome: microcephaly, developmental delay, intellectual disability, neurodegeneration in childhood	Autosomal recessive	Homozygous or compound heterozygous	*EIF2AK3* (also known as *PERK*)	R587Q, E331X, R902X, IVS14 + 1G-A, K345fsTer1, Q523fsTer4	[122,124,140–142]

[a]Abbreviations: PPP1R15B, protein phosphatase 1 regulatory subunit 15B; EIF2S2, eukaryotic initiation factor 2 subunit 3; MEHMO, mental retardation, epileptic seizures, hypogenitalism, microcephaly, obesity; EIF2B, eukaryotic initiation factor 2B; EIF2AK3, eukaryotic translation initiation factor 2α kinase 3.

including physical trauma, fear, and illness [105,106]. Disease-causing mutations can affect the abundance, guanine exchange activity, and/or assembly of the eIF2B complex, any of which theoretically could reduce cellular translation activity by reducing the pool of eIF2–GTP that is available for ternary complex formation [105,107–109]. However, biochemical analyses have revealed no single mechanistic explanation for all cases of VWMD [105,110]. Instead, it is likely that a spectrum of outcomes occurs depending on the mutated subunit and the degree to which the eIF2B complex is compromised.

Because eIF2B is a key regulatory node in the ISR, and is also responsible for the regeneration of the GTP–eIF2 complex to enable translation initiation, mutations that cause VWMD are likely to interfere with ISR resolution and/or normal translation activity in the cell. The ISR can be activated in cells of the brain upon trauma [38,111] and in neuroinflammatory contexts [67–69], and progressive episodes of white matter loss following these insults are noted in patients with VWMD. This observation supports the idea that perturbed ISR induction or resolution could result from disease-causing mutations in *EIF2B* genes. Consistent with eIF2B mutations affecting the ISR, VWMD patient cells typically have normal levels of translation in the absence of stress, but at least in some contexts hyper-repress translation during stress, fail to fully induce the GADD34 protein, and therefore are defective in stress recovery [112]. The VWMD phenotype is likely to also be affected by alterations in the proteome of eIF2B mutant cells, as well as by defects in inducing key molecules that promote remyelination following injury [113–115].

In a disease related to VWMD, mutations in the gene encoding the third subunit of the eIF2 complex (*EIF2G*/*EIF2S3*), cause a severe X-linked intellectual disability called **MEHMO** (mental retardation, epileptic seizures, hypogonadism and hypogenitalism, microcephaly, and obesity) syndrome [116–118]. **MEHMO syndrome** is characterized by severe intellectual disability,

cognitive and motor delays, microcephaly, hypomyelination, obesity, and hypogenitalism, and many patients also suffer from non-autoimmune diabetes mellitus and epilepsy [118]. As in VWMD, a spectrum of phenotypes are caused by different *EIF2S3* mutations in MEHMO syndrome. Notably, illness-aggravated pathologies associated with MEHMO syndrome have been reported, as seen in many VWMD cases [118]. Specific mutations in *EIF2S3* dramatically reduce cell growth/viability, the fidelity of translation initiation (as measured using reporters that assess AUG start site selection versus UUG start-site selection), and cause increased stress-induced gene expression in yeast models, whereas other mutations do not [116,118]. Analysis of a single-patient fibroblast sample from a patient with MEHMO syndrome revealed elevated CHOP expression at the protein and mRNA levels, indicating that some mutations in *EIF2S3* may reduce eIF2 activity and cause a chronic stress state [118]. Because some disease-causing mutations in *EIF2S3* perturb the interaction of eIF2γ with eIF2Bβ, it is possible that constitutively reduced translation activity contributes to a chronic stress response in patient cells. Further studies should be undertaken to determine how the full range of *EIF2S3* mutations that cause MEHMO syndrome affect the ISR, and assess drugs targeting the ISR as therapeutic intervention strategies.

Mutations in eIF2α Kinases Lead to Human Diseases

Mutations in two of the eIF2α kinases also give rise to specific human pathologies that are not restricted to the nervous system. Recessive mutations in *GCN2* lead to familial pulmonary capillary hemangiomatosis [119], or in other patients to a related pulmonary veno-occlusive disease (PVOD) [120,121]. Although these are not neuronal dysfunctions, one anticipates that these patients may have altered neuronal properties given the changes in *GCN2* function. Similarly, mutations in PERK lead to **Wolcott–Rallison syndrome**, a rare autosomal recessive form of diabetes [122,123]. Wolcott–Rallison syndrome patients often display some intellectual deficiencies, illustrating the role of PERK in proper neuronal development and function [123,124]. A rare allele of PERK (*EIF2AK3*) has also been identified that increases the risk of the tauopathy progressive supranuclear palsy (PSP), although how this specific alteration of PERK leads to PSP is unclear [125]. A patient with Wolcott–Rallison syndrome who had neurodegeneration (neurofibrillary tangles reminiscent of tauopathies, FUS-positivity in neurons as observed in frontotemporal dementia, activation of astrocytes and Bergmann glial cells, and elevated microglia) in childhood was homozygous for a premature stop codon mutation in *PERK* that could encode an inactive truncated form of PERK [124]. The observation that loss of these eIF2α kinases leads to genetic disease again illustrates the importance of proper eIF2 control for health and function.

Mutations in Phosphatases Targeting eIF2α

A final set of disease mutations affecting the phosphorylation of eIF2α are recessive mutations in the phosphatase gene *PPP1R15B*, also known as CReP (constitutive reverter of eIF2α phosphorylation). Like the stress-induced GADD34 protein, CReP is a regulatory subunit of PP1C that functions to dephosphorylate eIF2 when phosphorylated at Ser51. However, CReP is constitutively expressed [126]. These patients develop a multisystem syndrome with diabetes, microcephaly, intellectual disability, hypomyelination, and short stature [127,128], in line with the observation that *Ppp1r15b* deficiency severely impairs the development and growth of mice [129]. Importantly, disease-causing mutations reduce the phosphatase activity of CReP *in vitro* in rat insulin-secreting pancreatic β cell lines [127], and patient-derived cell lines show increased levels of p-eIF2α [128]. Expression of disease-causing CReP mutants promoted apoptosis in rat primary and insulinoma pancreatic β cell lines [127], and this could contribute to diabetes observed in this syndrome, potentially resulting from chronic and prolonged eIF2α phosphorylation. Therefore, defects in the regulators of the ISR can also contribute to human neurodevelopmental disease.

Box 1. Clinician's Corner

The control of translation and its regulation in response to stress is a crucial aspect of neuronal function. A key node in translational control is eIF2α phosphorylation, which downregulates bulk translation and allows selective translation of key mRNAs.

Phosphorylation of eIF2α is crucial in neuronal function and impacts synaptic plasticity as well as being inappropriately increased in numerous neurodegenerative diseases.

Mutations in several proteins involved in controlling the eIF2α phosphorylation circuit give rise to human diseases including neurological pathologies.

Compounds targeting the eIF2α kinases or phosphatases, or abrogating the effect of eIF2α phosphorylation, are in development. In model systems of neurological disease with perturbed eIF2α function, therapeutic restoration of proper eIF2α control can decrease the severity of disease.

In the future, pharmacological manipulation of the control and extent of eIF2α phosphorylation may be a possible therapy for the treatment of some neurological diseases.

Outstanding Questions

What are the differences in individual cell types that lead to unique responses to perturbation of eIF2α phosphorylation? The eIF2α kinases and phosphatases are widely expressed, but perturbation of the pathway often leads to defects in specific cell types for an unknown reason.

What additional regulatory circuits impinge upon components of eIF2α regulation, and how might they be manipulated for therapeutic benefit? The functional levels of the eIF2B complex affect the response to eIF2α phosphorylation, but how eIF2B is functionally controlled has not been fully elucidated.

Can effective therapies be developed that use small molecules to restore proper regulation of eIF2α phosphorylation in disease settings? This is a challenge because eIF2α phosphorylation is crucial for normal neuronal function, and a balance will be needed between preventing aberrant control through this regulatory site and maintaining its normal functions.

Concluding Remarks and Future Perspectives

Key questions remain in the field of eIF2α-dependent stress-responsive translation regulation and neuronal disorders (see Outstanding Questions). First, how different cell types and tissue contexts affect the sensitivity to acute and chronic stressors that activate eIF2α phosphorylation and the ISR, and the diversity in stress-induced gene expression, remain to be fully explored. Such an analysis could yield insight into why defects in the ISR seem to consistently cause neuronal (and often pancreatic) defects. Much research has revealed the main players in the ISR, including the eIF2α kinases and phosphatases, and has illuminated many of the stress-induced genes that are expressed following eIF2α phosphorylation. However, whether stress-induced genes are primarily regulated transcriptionally or post-transcriptionally during and after the ISR, and how cell type and disease context alters the identity of these genes, remains to be characterized. Third, future work should focus on the interplay between stress-induced RNA–protein granules and translation regulation during the ISR. Such research could shed light on the mechanisms by which repeat-expansion disorders and TDP-43 (another prion-like RNA-binding protein) mutations confer pathogenesis. Finally, exciting developments have resulted in the elucidation of several compounds (e.g., ISRIB, salubrinal, and PERK inhibitors) that allow us to chemically modulate essential components of the ISR. The utility of these compounds in alleviating a wide variety of neurodevelopmental and neurodegenerative diseases is only now beginning to be appreciated (Box 1). As future research identifies different disease contexts for which these compounds may be effective, tests these compounds in cell culture and animal models, and determines mechanisms for cell- and/or tissue-specific delivery of these compounds, much will be learned about the potential for therapeutic modulation of the ISR in many disease contexts.

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