REVIEW ARTICLE

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Messenger RNA Translation Defects in Neurodegenerative Diseases

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Neurodegenerative diseases are commonly adult-onset conditions characterized by degeneration of specific neuronal populations. Familial forms are caused by mutations in single genes, but most patients have a sporadic form that lacks a clear correlative mutation. Many neurodegenerative diseases are characterized by abnormal protein aggregates with a unique molecular composition for each disease. The molecular pathogenesis of neurodegenerative diseases remains largely enigmatic. Consequently, effective disease-modifying treatments are generally lacking.

Aberrant messenger RNA (mRNA) translation is implicated in a range of neurodegenerative diseases, including Alzheimer's disease, frontotemporal dementia, Parkinson's disease, Huntington's disease, prion disease, amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, and Charcot–Marie–Tooth disease (Table 1). Three different mechanisms are involved: global inhibition of translation, but with increased translation of a subset of mRNAs owing to activation of the "integrated stress response"; direct interaction between disease-associated proteins and the translation machinery; and mutations in genes encoding proteins that play a role in translation, which result in familial forms of neurodegenerative disease.

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Integrated Stress Response in Neurodegenerative Diseases

The integrated stress response is a signaling pathway that allows cells to cope with cellular stress by altering gene expression.[1] Many different stressors may activate this response. A hallmark of the integrated stress response is phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2), mediated by one of four eIF2α kinases that act as sensors of distinct forms of cellular stress (Fig. 1). Three eIF2α kinases have been implicated in neurodegenerative diseases: PERK, GCN2, and PKR. PERK is activated by unfolded or misfolded proteins in the endoplasmic reticulum, which cause endoplasmic reticulum stress, and is the predominant kinase that phosphorylates eIF2α at the basal level in the brain.[2] GCN2 is activated by ribosome collisions that are caused by depletion of aminoacylated transfer RNAs (tRNAs)[3-5] (e.g., as a consequence of amino acid deprivation). PKR is activated by double-stranded RNA, typically originating during viral infection. Recently, microtubule affinity–regulating kinase 2 (MARK2) was proposed as a fifth eIF2α kinase, activated by proteotoxic stress.[6] Specifically, HSP90 interacts with PKCδ, and misfolded proteins induce HSP90–PKCδ dissociation and PKCδ phosphorylation. Activated PKCδ phosphorylates and activates MARK2, leading to increased eIF2α phosphorylation and ATF4 induction.[6] The physiological relevance of this mechanism awaits confirmation. Other stressors such as oxidative stress, heat shock, and ultraviolet irradiation also activate one or more eIF2α kinases.[1]

The ternary complex of eIF2 (consisting of α, β, and γ subunits) with guanosine

Table 1. Translation Defects in Neurodegenerative Diseases.*

Disease	Gene	Nervous System	Neuronal Populations Affected	Other Cell Types Involved†	Effect on Translation	Activation and Effect of Integrated Stress Response‡
Alzheimer's disease	*APP*, *PSEN1*, *APOE4*	Central	Hippocampal and cortical neurons	Microglia, astrocytes	*APP*, *PSEN1*: inhibited; *APOE4*: not reported	Yes, detrimental
Frontotemporal dementia	*MAPT*	Central	Cortical neurons	Microglia, astrocytes	Inhibited	Yes, detrimental
Prion disease	—	Central	Broad neurodegeneration	Microglia, astrocytes	Inhibited	Yes, detrimental
Charcot–Marie–Tooth disease type 1B	*MPZ*	Peripheral	Peripheral motor and sensory neurons	Schwann cells	Not reported	Yes, protective
Charcot–Marie–Tooth disease type 2	*GARS1*, *YARS1*	Peripheral	Peripheral motor and sensory neurons	—	Inhibited	Yes, detrimental
Amyotrophic lateral sclerosis	*SOD1*, *FUS*, *C9orf72*	Central and peripheral	Upper and lower motor neurons	Microglia, astrocytes, oligodendrocytes	*SOD1*: not reported; *FUS*: inhibited; *C9orf72*: inhibited by DPR proteins	*SOD1*: yes, protective; *FUS*: yes, unknown; *C9orf72*: yes, detrimental
Huntington's disease	*HTT*	Central	Striatal and cortical neurons	Astrocytes, oligodendrocytes, microglia	Debated	Yes, effect unclear
Spinal muscular atrophy	*SMN1*	Peripheral	Lower motor neurons	Skeletal-muscle fibers	Inhibited	Not reported
Parkinson's disease	*LRRK2*, *PINK1*, *PRKN*	Central	Substantia nigra dopaminergic neurons	Microglia, astrocytes	*LRRK2*: increased; *PINK1*, *PRKN*: inhibited	*LRRK2*: not reported; *PINK1*, *PRKN*: yes, detrimental
Vanishing white-matter disease	*EIF2B1*, *EIF2B2*, *EIF2B3*, *EIF2B4*, *EIF2B5*	Central	White-matter axonopathy	Oligodendrocytes, astrocytes	Not changed in unstressed conditions	Yes, effect unclear

* *APOE4* denotes the ε4 allele of *APOE*, which encodes apolipoprotein E, *APP* the gene that encodes amyloid beta precursor protein, *C9orf72* the gene that encodes the C9orf72–SMCR8 complex subunit, DPR dipeptide repeat, *EIF2B1* through *EIF2B5* the genes that encode eukaryotic translation initiation factor 2B subunits 1 through 5, *FUS* the gene that encodes FUS RNA-binding protein, *GARS1* the gene that encodes glycyl-tRNA synthetase 1, *HTT* the gene that encodes huntingtin, *LRRK2* the gene that encodes leucine-rich repeat kinase 2, *MAPT* the gene that encodes microtubule-associated protein tau, *MPZ* the gene that encodes myelin protein zero, *PINK1* the gene that encodes PTEN-induced kinase 1, *PRKN* the gene that encodes parkin, *PSEN1* the gene that encodes presenilin 1, *SMN1* the gene that encodes survival of motor neuron 1, *SOD1* the gene that encodes superoxide dismutase 1, and *YARS1* the gene that encodes tyrosyl-tRNA synthetase 1.

† Other cell types are those implicated in neurodegeneration through non–cell-autonomous mechanisms.

‡ The effect of activation of the integrated stress response refers to the effect on neurodegeneration, neurodegenerative disease symptoms, or both.

triphosphate (GTP) and the initiator tRNA ($tRNA_i^{Met}$) binds to the 40S ribosomal subunit on the mRNA to facilitate interaction with the AUG initiation codon.[7] On AUG recognition, GTP is hydrolyzed to guanosine diphosphate (GDP). The guanine nucleotide exchange factor eIF2B catalyzes the nucleotide exchange of eIF2-GDP into eIF2-GTP, which is rate limiting for ternary complex formation and function. A decameric (10-component) complex, eIF2B is composed of two heteropentamers consisting of α, β, γ, δ, and ε subunits, of which the ε subunit harbors the catalytic guanine nucleotide exchange activity.[1] Whereas nonphosphorylated eIF2 is a substrate for eIF2B, phosphorylation of eIF2α converts eIF2 from an activator into an inhibitor of eIF2B (Fig. 2A). Thus, eIF2α phosphorylation causes inhibition of global mRNA translation by reducing the amount of the ternary complex.

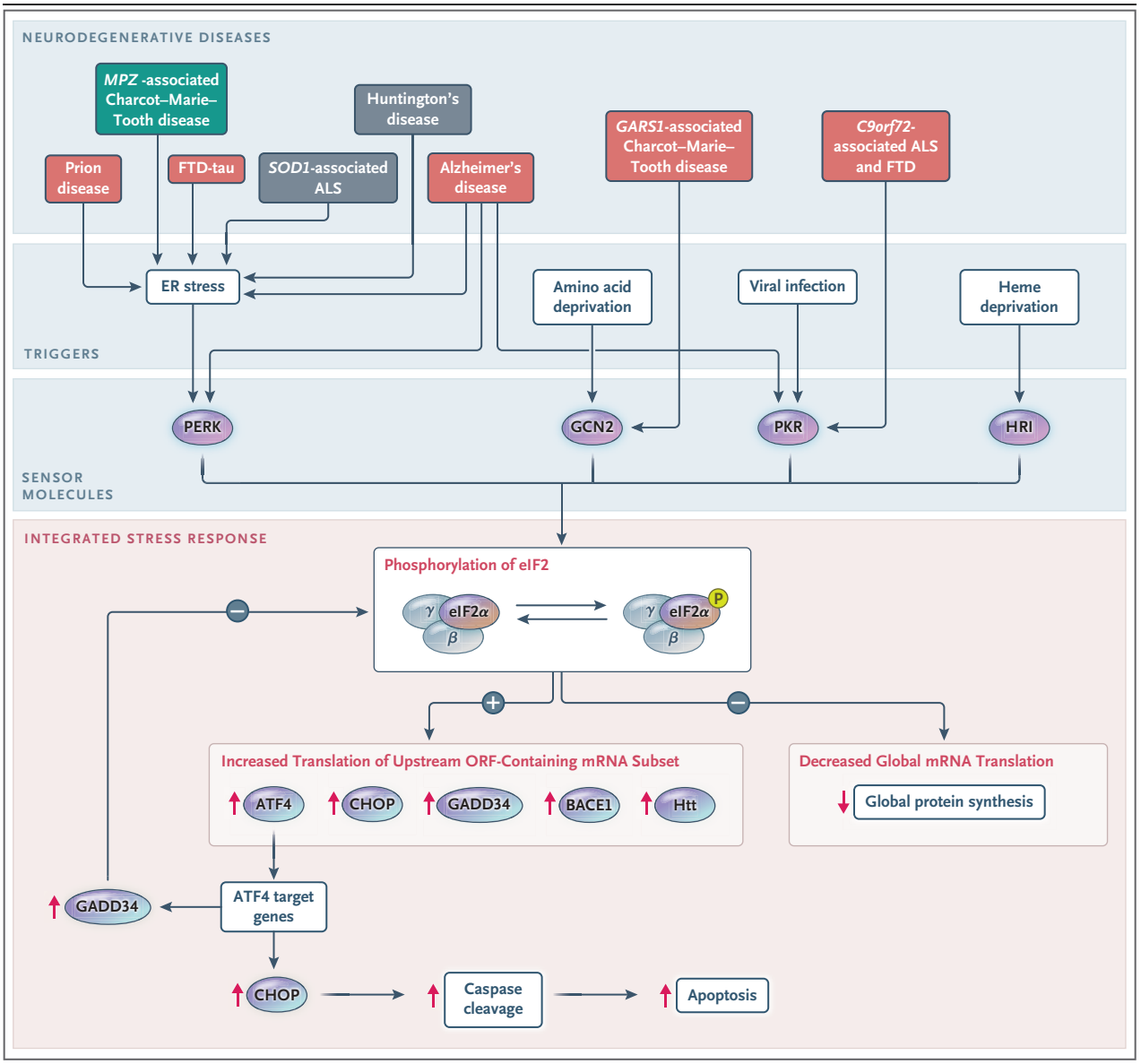
Paradoxically, eIF2α phosphorylation increases the translation of a subset of mRNAs that have upstream open reading frames (ORFs) in their 5′ untranslated region (UTR).[7] Upstream ORFs can suppress translation of the main ORF through various mechanisms.[8] When the ternary complex concentration is limited as a result of activation of the integrated stress response, the 40S ribosome scans through the initiation codon of the upstream ORF, allowing it to reach the main ORF (Fig. 2B). Upstream ORF–containing mRNAs include ATF4, C/EBP homologous protein (CHOP), and GADD34 (Fig. 1). ATF4 is a transcription factor with target genes that include chaperones and proteases that bolster protein folding and degradation.

Attenuating cellular proteostasis in response to stress is beneficial. However, prolonged activation of the integrated stress response, as has been documented in many neurodegenerative diseases, is harmful.[1] Increased brain levels of phosphorylated eIF2α, PERK, PKR, or GCN2 have been reported in postmortem studies involving patients with Alzheimer's disease, frontotemporal dementia, Parkinson's disease, Huntington's disease, ALS, and Charcot–Marie–Tooth disease and in mouse models of these diseases.[1] In several of these disorders, genetic and pharmacologic manipulations in animal models have shown that activation of the integrated stress response is not just an epiphenomenon of neurodegeneration but actively promotes disease.

Alzheimer's Disease

Phosphorylation of eIF2α is elevated in the brains of patients with Alzheimer's disease and in mouse models of familial Alzheimer's disease.[9-11] Genetic deletion of PERK in excitatory neurons in mouse models of familial Alzheimer's disease has resulted in diminished eIF2α phosphorylation, relief of inhibited global protein synthesis, and, strikingly, correction of deficits in synaptic plasticity and spatial memory.[9] These findings are consistent with a role of eIF2α phosphorylation in long-term synaptic plasticity and memory.[12] In a different mouse model of familial Alzheimer's disease, PERK haploinsufficiency not only rescued memory deficits by reducing eIF2α phosphorylation and ATF4 up-regulation but also reduced cholinergic neurodegeneration.[10] Elevated eIF2α phosphorylation also resulted in increased translation of the mRNA encoding BACE1 (β-site amyloid precursor protein–cleaving enzyme 1), which contains three upstream ORFs in its 5′ UTR.[13] BACE1 is a protease with amyloid precursor protein (APP) β-secretase activity, which catalyzes the first step of amyloid-beta (Aβ) generation.[14] In the above-mentioned mouse model of familial Alzheimer's disease, reduced eIF2α phosphorylation through PERK haploinsufficiency consistently prevented elevation of BACE1 levels and reduced Aβ peptide levels, as well as the plaque burden, a pathological hallmark of Alzheimer's disease.[10]

PKR is also implicated in the pathogenesis of Alzheimer's disease. In mice and monkeys, intracerebroventricular infusion of Aβ oligomers resulted in increased brain tumor necrosis factor α (TNF-α) levels, leading to TNF receptor 1 (TNFR1)–mediated PKR phosphorylation and increased eIF2α phosphorylation, along with synapse loss and memory impairment.[11] In mice with loss of PKR or TNFR1 function, Aβ oligomers failed to trigger eIF2α phosphorylation and cognitive impairment.[11] Similarly, in a genetic mouse model of familial Alzheimer's disease, loss of PKR led to a decrease in BACE1 levels, which was associated with improved memory consolidation and reductions in brain Aβ levels and plaque load, apoptosis, synapse loss, and neuroinflammation.[15] In a transgenic mouse model expressing human apolipoprotein E4, eIF2α phosphorylation and ATF4 levels were increased in the hippocampus, and pharmacologic PKR inhibition reduced ATF4 levels and



corrected the cognitive impairment.[16] This finding substantiates the role of PKR-mediated activation of the integrated stress response in sporadic Alzheimer's disease.

FRONTOTEMPORAL DEMENTIA

Hyperactivation of PERK has been implicated in frontotemporal dementia associated with pathological inclusions of hyperphosphorylated tau (FTD-tau). In an FTD-tau mouse model, increased levels of phosphorylated eIF2α and ATF4 were accompanied by repression of global protein synthesis at the onset of neurodegeneration.[17] Treatment with GSK2606414, a small-molecule PERK inhibitor, reduced phosphorylated eIF2α levels, restored protein synthesis, and mitigated neuronal loss and brain atrophy.[17]

PRION DISEASE

In mice with prion disease, accumulation of prion protein in the endoplasmic reticulum induces the integrated stress response, causing increases in levels of phosphorylated PERK, phosphorylated eIF2α, CHOP, and cleaved caspase 12, as well as repression of protein synthesis.[18] Overexpression of GADD34 — the regulatory subunit of the PP1–GADD34 phosphatase complex, which selectively dephosphorylates

Figure 1 (facing page). The Integrated Stress Response and Neurodegenerative Diseases.

The integrated stress response converges on phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2), mediated by four kinases: PERK, GCN2, PKR, and HRI, which act as sensor molecules for distinct types of cellular stress. PERK, which is activated by unfolded or misfolded proteins in the endoplasmic reticulum (ER), is a transmembrane protein with its regulatory domain in the ER lumen and its kinase domain on the cytoplasmic side. GCN2 is activated by amino acid deprivation, resulting in depletion of aminoacylated transfer RNAs (tRNAs), leading to stalling of translating ribosomes and ribosome collisions. This results in GCN2 recruitment to ribosomes and GCN2 activation. PKR is activated by double-stranded RNA, typically originating from viral infection. HRI is primarily expressed in erythrocytes, activated by heme deprivation, and less relevant in the context of neurodegenerative diseases. Beyond the "canonical" stressors, other forms of cellular stress such as oxidative stress, heat shock, ultraviolet irradiation, and cytosolic protein aggregation also activate one or more eIF2α kinases, resulting in activation of the integrated stress response. Detection of stress signals by the regulatory domains of the eIF2α kinases triggers kinase activation by dimerization and trans-autophosphorylation. Phosphorylation of eIF2α inhibits global messenger RNA (mRNA) translation but increases translation of a subset of mRNAs, including mRNAs encoding the transcription factors ATF4 and C/EBP homologous protein (CHOP), GADD34, the regulatory subunit of the PP1–GADD34 phosphatase, which selectively dephosphorylates eIF2α, β-site amyloid precursor protein–cleaving enzyme 1 (BACE1), and Huntingtin (Htt). ATF4 target genes include *GADD34* and *DDIT3* (which encodes CHOP). Induction of GADD34 expression acts as a feedback loop that terminates activation of the integrated stress response when cellular stress is resolved. Failure to mitigate cellular stress causes activation of CHOP, the target genes of which engender caspase cleavage and apoptosis. At the top of the figure, the reddish brown boxes indicate a deleterious effect of activation of the integrated stress response in neurodegenerative diseases, the green box a protective effect, and the gray boxes a debated effect. ALS denotes amyotrophic lateral sclerosis, FTD-tau frontotemporal dementia with pathological tau inclusions, *GARS1* the gene that encodes glycyl-tRNA synthetase 1, *MPZ* the gene that encodes myelin protein zero, ORF open reading frame, and *SOD1* the gene that encodes superoxide dismutase 1.

eIF2α — or pharmacologic inhibition of PERK by GSK2606414 reduces phosphorylated eIF2α levels, restores translation rates, corrects synaptic deficits and neuronal loss, and increases survival.[18,19] Conversely, increasing phosphorylated eIF2α levels through pharmacologic inhibition of PP1–GADD34 exacerbates neurotoxicity and reduces survival among mice with prion disease.[18] Strikingly, selective GADD34 overexpression in hippocampal neurons[18] or in astrocytes[20] prevents neuronal loss, reduces astrogliosis, and prolongs survival among prion-infected mice. The contribution of astrocytes to synaptic toxicity has been attributed to alteration of the astrocytic secretome induced by prion-mediated activation of the integrated stress response.[20]

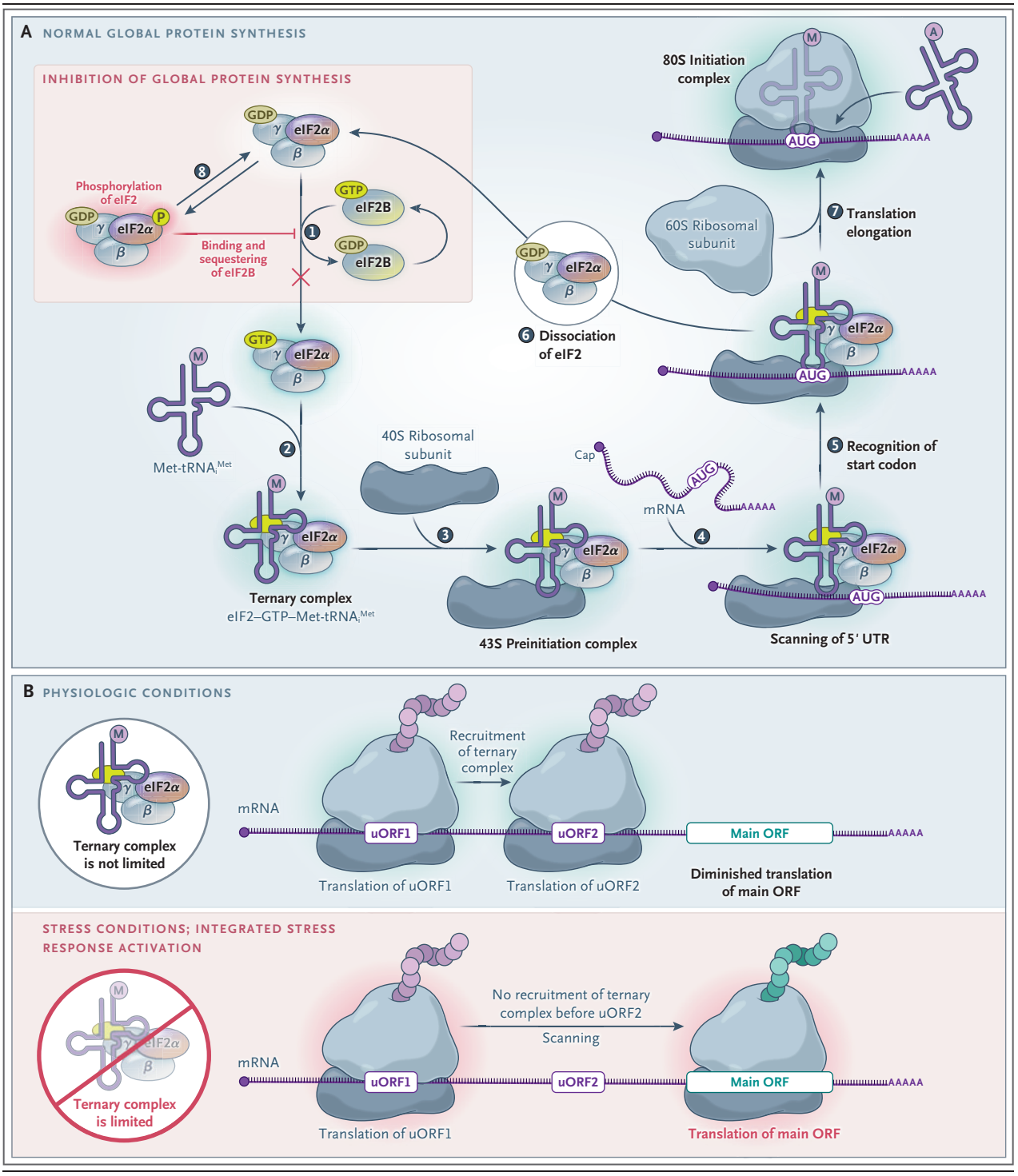
CHARCOT–MARIE–TOOTH DISEASE

Although animal models of Alzheimer's disease, FTD-tau, and prion disease have shown that reducing eIF2α phosphorylation is beneficial, the opposite has been reported for Charcot–Marie–Tooth disease type 1B (CMT1B), a form of Charcot–Marie–Tooth disease characterized by demyelinating peripheral neuropathy due to dominantly inherited mutations in *MPZ*, encoding myelin protein zero. Charcot–Marie–Tooth disease, which is characterized by degeneration of peripheral motor and sensory axons, can develop at any age from infancy through adulthood. In a CMT1B transgenic mouse model with mutant MPZ expression in which serine 63 was deleted (MPZ-S63del), misfolded MPZ-S63del protein was retained in the endoplasmic reticulum, resulting in activation of the integrated stress response in Schwann cells.[21] Inactivation of GADD34 in MPZ-S63del mice increases eIF2α phosphorylation and ATF4 levels, restores motor function, and ameliorates neurophysiological and neuropathological defects, including hypomyelination.

In agreement with this finding is the observation that treatment of MPZ-S63del mice with Sephin1, a small-molecule selective GADD34 inhibitor, corrected motor deficits and peripheral axon myelination.[22] Both genetic mutation[21] and pharmacologic inhibition[22] of GADD34 limited reactivation of translation and attenuated the integrated stress response. Conversely, MPZ-S63del mice in which eIF2α cannot be phosphorylated in Schwann cells have more pronounced demyelination and reduced nerve-conduction velocity.[23] In contrast to these findings, activation of the integrated stress response is detrimental in Charcot–Marie–Tooth disease caused by mutations in *GARS1*, encoding glycyl-tRNA synthetase.

AMYOTROPHIC LATERAL SCLEROSIS

Expansion of the GGGGCC hexanucleotide repeat in the first intron of *C9orf72* is the most



prevalent genetic cause of ALS and frontotemporal dementia.[24,25] Repeat-associated non-AUG (RAN) translation of the expanded GGGGCC repeat results in the production of toxic dipeptide repeat proteins.[26] Phosphorylation of eIF2α, the small ribosomal protein subunit 25, and eIF2D are involved in RAN translation.[27-30] The PKR pathway plays a key role in promoting RAN translation because expanded nucleotide repeat RNAs activate PKR, probably through formation

Figure 2 (facing page). Regulation of mRNA Translation Initiation.

Panel A shows that increased eIF2α phosphorylation inhibits global mRNA translation. The guanine nucleotide exchange factor (GEF) eIF2B converts eIF2–guanosine diphosphate (GDP) into eIF2–guanosine triphosphate (GTP) (step 1), which associates with methionyl-initiator tRNA (Met-tRNA$_i^{Met}$) to form the ternary complex (step 2). The ternary complex binds to the 40S small ribosomal subunit, along with other initiation factors (not shown), to form the 43S preinitiation complex (step 3). The preinitiation complex is subsequently recruited to the mRNA 5′ cap structure by the cap-binding eIF4F complex (not shown) and scans the 5′ untranslated region (UTR) until it reaches an AUG start codon (step 4). AUG recognition triggers GTP hydrolysis, which facilitates Met-tRNA$_i^{Met}$ base pairing with the AUG codon (step 5) and subsequent eIF2-GDP dissociation (step 6). This allows recruitment of the 60S large ribosomal subunit to form the 80S initiation complex, which starts the elongation phase of translation (step 7). The α, β, and γ subunits compose eIF2 (step 8). Whereas nonphosphorylated eIF2 is a substrate for eIF2B GEF activity, phosphorylation of eIF2 converts it into an inhibitor of eIF2B, thus limiting eIF2B activity and reducing the cellular ternary complex concentration, leading to inhibition of global translation. Panel B shows an mRNA with two upstream ORFs (uORFs) preceding the main ORF. In physiologic (unstressed) conditions, the cellular ternary complex concentration is not limited. Consequently, after translating uORF1, the ribosome recruits a ternary complex during scanning before it reaches uORF2, resulting in uORF2 translation, which in turn diminishes translation of the main ORF. Under stress conditions, the ternary complex concentration is limited because of activation of the integrated stress response, which typically leads to failure of the ribosome to recruit a ternary complex during scanning before reaching uORF2, resulting in scanning through uORF2 and translation of the main ORF.

of double-stranded RNA structures.[31] Studies have consistently shown that increasing the cellular ternary complex concentration by means of an integrated stress response inhibitor, which negates the deleterious effect of eIF2α phosphorylation by activating eIF2B,[1] or pharmacologically inhibiting eIF2α phosphorylation, reduces RAN translation.[28,29,31] Treatment of a mouse model of *C9orf72*-associated ALS or frontotemporal dementia with metformin and other biguanides, which inhibit PKR, decreases dipeptide repeat proteins and ameliorates behavioral and neuropathological deficits.[31] The mechanism through which limited ternary complex levels promote RAN translation remains to be determined.

In a mouse model of ALS associated with mutations in *FUS* RNA-binding protein, mutant FUS accumulated in axons but not in cell bodies of cultured hippocampal neurons and in sciatic nerve axons in vivo.[32] This accumulation correlated with increased phosphorylated eIF2α levels and suppression of global protein synthesis in axons but not in cell bodies at the onset of early symptoms.[32] The mechanism underlying axonal accumulation of mutant FUS, the eIF2α kinase that elicits a local integrated stress response, and the trigger for its induction remain to be elucidated.

In three mouse models of superoxide dismutase 1 (*SOD1*)–associated ALS, the PERK arm of the integrated stress response was activated in fast-fatigable motor neurons — the motor neuron subpopulation that degenerates first[33] — in presymptomatic mice, weeks before denervation and axonal degeneration occurred.[34] In contrast, the stress response was not activated in slow motor neurons, which are resistant to degeneration.[34] Translatome analysis in motor neurons, astrocytes, and oligodendrocytes from SOD1-G37R mice corroborated the selective PERK activation in motor neurons, which may be attributable to low levels of endoplasmic reticulum chaperones in motor neurons, as compared with the levels in astrocytes and oligodendrocytes.[35] Misfolded SOD1 also induces PKCδ–MARK2–eIF2α signaling, and increased PKCδ and MARK2 phosphorylation was documented in a SOD1-ALS mouse model and in patients with ALS.[6]

In support of a protective effect of enhanced eIF2α phosphorylation, pharmacologic inhibition of PP1-GADD34–mediated eIF2α dephosphorylation with the use of salubrinal[34] or Sephin1[22] delayed the onset of disease, ameliorated motor deficits, delayed denervation and motor neuron loss, and extended the life span in SOD1-G93A mice. This may be explained by reduced mutant SOD1 aggregation, which may alleviate endoplasmic reticulum stress and activation of the stress response.[22] However, the role of activation of the integrated stress response in the pathogenesis of *SOD1*-associated ALS remains controversial, and the findings of another study argue against it.[36] Recently, the effect of guanabenz, an α_2-adrenoreceptor agonist identified as a GADD34 inhibitor,[37] in patients with sporadic ALS was evaluated in a phase 2 clinical trial.[38] The results were encouraging, with high doses of guanabenz resulting in slower disease progression at

6 months. To avoid hypotensive side effects, alternative GADD34 inhibitors that lack α_2-adrenergic activity should be tested in clinical trials.

MECHANISM OF INTEGRATED STRESS RESPONSE ACTIVATION

Several neurodegenerative disease–associated proteins are targeted to the endoplasmic reticulum and enter the secretory pathway. These include APP and the β- and γ-secretase complexes, prion protein, and MPZ. Misfolding of these proteins or derived peptides is thought to activate the integrated stress response.[1] ALS-mutant SOD1 proteins may activate the stress response through aberrant interaction with the endoplasmic reticulum transmembrane protein Derlin-1, which is involved in the endoplasmic reticulum–associated degradation (ERAD) pathway.[39] Normally, ERAD substrates (misfolded proteins) are transported from the endoplasmic reticulum lumen to the cytoplasm through a retro-translocation channel, followed by degradation through the ubiquitin–proteasome pathway. ALS-mutant SOD1 protein is thought to form a complex with Derlin-1 and VIMP (VCP-interacting membrane protein), which traps ERAD substrates on the cytoplasmic side of the retro-translocation channel, inhibiting their subsequent ubiquitination. This results in ERAD inhibition and accumulation of misfolded proteins in the endoplasmic reticulum lumen, which triggers the stress response.[39] Pathogenic huntingtin (Htt) oligomers similarly cause ERAD inhibition and stress response induction.[40,41] As a result of an upstream ORF in the 5′ UTR of the Htt mRNA that inhibits Htt translation,[42] integrated stress response induction enhances Htt translation and aggregation, thus seeding a vicious cycle.[43]

INTERACTION OF MUTANT PROTEINS WITH MRNA TRANSLATION MACHINERY

Several neurodegeneration-associated proteins, including tau, dipeptide repeat proteins, Htt, and survival motor neuron (SMN) protein, the loss of which causes the neuromuscular disease spinal muscular atrophy, have been reported to bind to ribosomes. Pathological tau associates with ribosomes in the brains of patients with Alzheimer's disease and impairs translation in cultured cells.[44] Furthermore, global protein synthesis is decreased in the cortex and hippocampus in mouse models of tauopathy.[45]

In several studies, *C9orf72*-ALS and *C9orf72*–frontotemporal dementia–associated poly–glycine–arginine (poly-GR) and poly–proline–arginine (poly-PR) dipeptide repeat proteins expressed in cultured motor neurons and astrocytes and in mouse and fly brains bound to ribosomal proteins and inhibited global protein synthesis.[46-49] Furthermore, in the brains of patients with ALS or frontotemporal dementia associated with *C9orf72*, ribosomal proteins colocalized with dipeptide repeat protein inclusions, indicating that sequestration of ribosomes in these aggregates may lead to inhibition of global protein synthesis.[47,48] Mechanistically, poly-PR and poly-GR block the polypeptide tunnel of the ribosome, extending into the peptidyl transferase center and inhibiting its activity.[50]

In cellular and mouse models of Huntington's disease, the interactome of soluble Htt oligomers is enriched for proteins of the ribosome biogenesis and mRNA translation apparatuses.[51,52] Htt interacts with ribosomal proteins, and the polyglutamine (polyQ)–expanded Htt has a stronger interaction than wild-type Htt.[52,53] This observation correlates with the observed inhibition of global protein synthesis in cellular models of Huntington's disease[53] and dose-dependent inhibition of translation by both wild-type and mutant Htt in in vitro translation systems.[52,53] Beyond persistent activation of the integrated stress response,[40,41] suggested mechanisms for the Htt-mediated inhibition of translation include idling of ribosomes during the elongation phase[53] and defective ribosome biogenesis.[51] Recently, polyQ-expanded Htt was also reported to sequester eIF5A, and eIF5A depletion from ribosomes promoted widespread ribosome stalling on hundreds of transcripts.[43]

At variance with the aforementioned findings is a report of elevated global protein synthesis in the striatum of two mouse models of Huntington's disease, starting from the age at which symptoms developed.[54] This elevation was attributed to increased phosphorylation of the translational repressor eIF4E-binding protein (4E-BP1), which results in dissociation of 4E-BP1 from the cap-binding protein eIF4E and alleviation of repression.[55] The level of the eIF4F complex (consisting of eIF4A, eIF4E, and eIF4G) was increased in the striatum in a mouse model of Huntington's dis-

ease. Furthermore, intracerebroventricular infusion of 4EGI-1, a small-molecule inhibitor that prevents eIF4E–eIF4G association, normalizes eIF4F complex levels and protein synthesis and ameliorates motor deficits.[54] To resolve the discrepancy between increased translation (initiation) in striatum in a mouse model of (early) symptomatic Huntington's disease and inhibition of translation (elongation) in cellular models, the dependency of these effects on disease stage and the possible contribution of astrogliosis and microgliosis should be investigated. Additional methods to evaluate translation in striatum in mice with Huntington's disease should be used to rule out possible technical issues with puromycin incorporation assays.

SMN associates with translating ribosomes in a tissue-specific and RNA-independent manner to stimulate translation. SMN-bound ribosomes are preferentially positioned within the first five codons of a subset of mRNAs.[56] Loss of SMN reduced ribosome occupancy, especially at the beginning of the coding sequence of SMN-associated mRNAs,[56] and inhibited protein synthesis in motor neurons and in the brain and spinal cord in a mouse model of spinal muscular atrophy at late symptomatic stages.[57,58]

Ribosomal protein S15 (RPS15) is a substrate of the LRRK2 kinase carrying the Parkinson's disease–associated G2019S mutation in dopaminergic neurons in drosophila and human models of Parkinson's disease.[59] The mutation enhances LRRK2 activity. Although LRRK2 G2019S expression in drosophila stimulates global protein synthesis, expression of nonphosphorylatable RPS15 normalizes protein synthesis and ameliorates dopaminergic neurodegeneration and age-dependent locomotor deficits. Similarly, expression of a nonphosphorylatable RPS15 in LRRK2 G2019S human dopaminergic and cortical neurons mitigates neurite loss and cell death, showing that RPS15 phosphorylation has a causal role in neurodegeneration.[59] Ribosome profiling of LRRK2 G2019S dopaminergic neurons derived from human induced pluripotent stem cells revealed that, as compared with controls, some mRNAs were translated more efficiently, including those encoding voltage-gated Ca^{2+} channels, leading to increased Ca^{2+} influx and elevated Ca^{2+} levels in human LRRK2 G2019S dopaminergic neurons.[60] The underlying mechanism remains to be elucidated.

Two additional proteins associated with autosomal recessive Parkinson's disease, the serine–threonine protein kinase PINK1 and the E3 ubiquitin ligase parkin, promote the translation of a subset of nuclear-encoded respiratory-chain component mRNAs at the mitochondrial outer membrane.[61] Consequently, the PINK1–parkin pathway regulates mitochondrial oxidative phosphorylation.[61] An enhanced association between defective mitochondria and the endoplasmic reticulum was found in drosophila Pink1 and parkin mutants and patient-derived *PINK1* and *PRKN* mutant fibroblasts. This association was mediated by mitofusin and resulted in PERK activation, increased levels of phosphorylated eIF2α, and inhibition of global protein synthesis.[62] Reducing the association between defective mitochondria and the endoplasmic reticulum by means of mitofusin knockdown or inhibition of PERK–eIF2α signaling corrected the protein synthesis defect and prevented dopaminergic neurodegeneration in drosophila Pink1 and parkin mutants.[62]

MUTATIONS IN GENES ENCODING MRNA TRANSLATION COMPONENTS

VANISHING WHITE-MATTER DISEASE

Autosomal recessive mutations in the genes encoding each of the five subunits of eIF2B cause leukoencephalopathy with vanishing white matter,[63,64] a chronic, progressive neurologic disorder with a childhood onset, characterized by cerebellar ataxia, spasticity, and cognitive impairment associated with white-matter lesions on brain imaging. Mutations that cause vanishing white-matter disease reduce eIF2B activity through various mechanisms, including reduced steady-state levels of the mutant subunit, impaired eIF2B complex formation or stability, reduced eIF2 binding, and direct impairment of guanine nucleotide exchange factor activity.[65-67]

Activation of the integrated stress response has been documented in the white matter in patients with vanishing white-matter disease and in a mouse model of the disease, predominantly in oligodendrocytes and astrocytes.[68,69] Furthermore, in lymphoblast cell lines derived from patients with vanishing white-matter disease, translational suppression during activation of the integrated stress response caused by acute endoplasmic reticulum stress was exaggerated.[70]

Pharmacologic interventions (an integrated stress response inhibitor [ISRIB] and the eIF2B activator 2BAct) that stabilize mutant eIF2B complexes in vanishing white-matter disease and restore their guanine nucleotide exchange activity ameliorated motor deficits, demyelination, and reactive astrogliosis and microgliosis in a mouse model of this disease.[69,71] These observations are consistent with a key role of decreased eIF2B guanine nucleotide exchange factor activity in the pathogenesis of vanishing white-matter disease.

IMBALANCE BETWEEN TRNA SUPPLY AND MRNA CODON DEMAND

Dominantly inherited mutations in seven genes encoding aminoacyl-tRNA *s*ynthetases (aaRSs) cause axonal and intermediate forms of Charcot–Marie–Tooth disease.[72,73] Aminoacylated tRNAs are delivered to the ribosome by elongation factor eEF1A (Fig. 3). Although many Charcot–Marie–Tooth disease–causing aaRS mutations result in loss of aminoacylation activity, others do not affect catalytic activity,[72,73] indicating that loss of aminoacylation activity is not required for the development of Charcot–Marie–Tooth disease. Nevertheless, expression of six distinct Charcot–Marie–Tooth disease–associated mutant variants of glycyl- or tyrosyl-tRNA synthetase, including aminoacylation-active variants, dramatically inhibits global protein synthesis in drosophila motor and sensory neurons, suggesting that defective translation constitutes a unifying pathogenic mechanism underlying aaRS-associated Charcot–Marie–Tooth disease.[74]

Charcot–Marie–Tooth disease–associated mutant glycyl-tRNA synthetase (GlyRS) variants bind $tRNA^{Gly}$ but are defective in releasing it.[75] Consequently, the cellular $tRNA^{Gly}$ pool is depleted, leaving insufficient $tRNA^{Gly}$ substrate for the wild-type GlyRS in heterozygous patients. This results in an insufficient supply of glycyl-$tRNA^{Gly}$ to the ribosome and stalling of the ribosome on Gly codons[75] (Fig. 3). Consistent with this model is the observation that transgenic $tRNA^{Gly}$ overexpression ameliorates translation defects and peripheral neuropathy phenotypes in drosophila and mouse models of *GARS1*-associated Charcot–Marie–Tooth disease.[75] Ribosome stalling activates GCN2,[3-5] resulting in selective induction of the integrated stress response in affected motor and sensory neurons.[76] Activation of the integrated stress response leads to peripheral neuropathy in mouse models of *GARS1*-associated Charcot–Marie–Tooth disease, which can be partially ameliorated by genetic inactivation or pharmacologic inhibition of GCN2.[76] The molecular mechanism underlying activation of the selective stress response in affected motor and sensory neurons warrants further investigation. Moreover, whether mutations in other aaRS-encoding genes also cause Charcot–Marie–Tooth disease through tRNA sequestration is an open question.

Whereas dominantly inherited mutations in genes encoding cytoplasmic aaRSs cause peripheral neuropathies, recessively inherited mutations in genes encoding cytoplasmic or mitochondrial aaRSs cause various syndromes, often involving neurologic symptoms. Although many of the recessive diseases have not been extensively studied, existing evidence indicates that a substantial loss of aminoacylation activity may reduce the levels of aminoacylated cognate tRNA, leading to translation defects in the cytoplasm or mitochondria.[77] Whether translation defects are attributable to ribosome stalling on cognate codons and GCN2-mediated activation of the stress response is currently unknown.

Loss of function of n-Tr20, a neuronally enriched $tRNA^{Arg}$, results in ribosome pausing on Arg codons, altered synaptic transmission, and reduced susceptibility to seizures; this finding corroborates the notion that tRNA depletion causes neurologic dysfunction and neurodegeneration.[78] When combined with loss of function of ribosome rescue factor GTPBP1 or GTPBP2, loss of n-Tr20 function results in neurodegeneration in mice.[79,80] Ribosome stalling on Arg codons also results in GCN2-mediated activation of the integrated stress response.[78,81] However, whereas GCN2-mediated activation promotes axonal degeneration in mouse models of *GARS1*-associated Charcot–Marie–Tooth disease,[76] this same pathway promotes neuronal survival in *n-Tr20*$^{-/-}$; *Gtpbp1/2*$^{-/-}$ mice.[81] This discrepancy warrants further investigation.

A similar tRNA supply–codon demand mismatch mechanism was identified in mice in which Nsun2, a tRNA cytosine methyltransferase that is essential for cytosine methylation in the variable loop of numerous tRNAs, was selectively inactivated in excitatory forebrain neurons.[82] Loss of *Nsun2* function resulted in a relatively selective reduction of $tRNA^{Gly}$ levels, leading

to excessive ribosome pausing on Gly codons, decreased expression of Gly-rich proteins, and synaptic transmission defects, culminating in cognitive deficits and depressive behavior.[82]

Finally, in a cellular model of Huntington's disease, translation of expanded CAG repeats in mutant Htt led to reduced levels of charged glutaminyl-tRNA$^{Gln\text{-}CUG}$, resulting in translational frameshifting on the CAG repeat stretch and the generation of hybrid polyglutamine–polyalanine proteins, which may nucleate polyQ aggregation. The frequency of frameshifting increased proportionally to the length of the CAG repeat stretch and was inversely correlated with glutamyl-tRNA$^{Gln\text{-}CUG}$ levels.[83] Ribosome profiling revealed that CAG repeats promote ribosome collisions on mutant Htt mRNA, resulting in increased incidences of premature termination of nascent Htt and release of aggregation-prone fragments.[43] Pharmacologic reduction of translation initiation attenuated ribosome collisions and mitigated ribotoxicity in a cellular model of Huntington's disease, underscoring the pathologic relevance of this mechanism.[43]

PONTOCEREBELLAR HYPOPLASIA

Pontocerebellar hypoplasia constitutes a group of rare autosomal recessive neurodegenerative disorders manifested as hypoplasia of the cerebellum and pons, progressive microcephaly, and variable neocortical atrophy.[84] Mutations in each of the four subunits of the tRNA splicing endonuclease (TSEN) complex, which mediates tRNA intron excision, result in pontocerebellar hypoplasia.[85,86] These mutations cause defects in TSEN complex assembly and stability[86] and an accumulation of pre-tRNAs in fibroblasts — surprisingly, without an obvious effect on mature tRNA levels.[87] Thus, pontocerebellar hypoplasia may result from the toxic effects of pre-tRNA accumulation or alteration of alternative TSEN functions, such as endonucleolytic cleavage of a subset of mRNAs, which initiates an mRNA decay pathway.[88]

A homozygous R140H mutation in the RNA kinase CLP1 (cleavage factor polyribonucleotide kinase subunit 1) also causes pontocerebellar hypoplasia.[89,90] CLP1 associates with TSEN and acts as a negative regulator of pre-tRNA splicing through phosphorylation of the tRNA 3′ exon, which prevents the tRNA ligase RTCB from producing the mature tRNA and may promote degradation of tRNA fragments.[91] Kinase activity of CLP1-R140H is defective, and the TSEN complex is destabilized as a result of loss of CLP1–TSEN interaction, leading to impaired tRNA cleavage and accumulation of pre-tRNAs and tRNA introns.[89,90] Mice that are homozygous for a kinase-dead CLP1 have pontocerebellar hypoplasia–like phenotypes and progressive loss of motor neurons.[90,92] Loss of CLP1 activity results in accumulation of pre-tRNA–derived fragments, which sensitize cells to oxidative stress–induced p53 activation and p53-dependent cell death.[89,92] Introduction of the R140H mutation in the mouse *Clp1* gene resulted in motor deficits that were due to the loss of lower motor neurons and cerebellar neurons.[93] Pre-tRNAs and tRNA introns accumulated in the mice, whereas levels of mature tRNAs were not affected. CLP1 also associates with the cleavage and polyadenylation machinery, and *Clp1-R140H* mice had pre-mRNA cleavage defects, with a shift from proximal to distal polyadenylation sites in a number of transcripts, typically leading to reduced expression levels. Thus, impaired mRNA 3′ processing may contribute to the pathogenesis of CLP1-associated pontocerebellar hypoplasia.

Finally, mutations in *AMPD2*, encoding adenosine monophosphate deaminase 2, constitute another genetic cause of pontocerebellar hypoplasia. AMPD2 is involved in maintenance of cellular guanine nucleotide pools, and AMPD2 deficiency results in defective GTP-dependent initiation of translation, which can be corrected by the administration of purine precursors.[94]

CONCLUSIONS

Translation defects are pervasive in neurodegenerative diseases. Genetic or pharmacologic modulation of translation in animal models mitigates disease-associated phenotypes and neurodegeneration. These findings indicate that translation defects are not merely a consequence of neurodegeneration but may instead actively contribute to disease progression or even trigger neurodegeneration. Inhibition of translation has been implicated in most neurodegenerative diseases, except for LRRK2-G2019S–associated Parkinson's disease, in which increased translation may confer toxic effects.

Long-term activation of the integrated stress response has been implicated in a remarkable

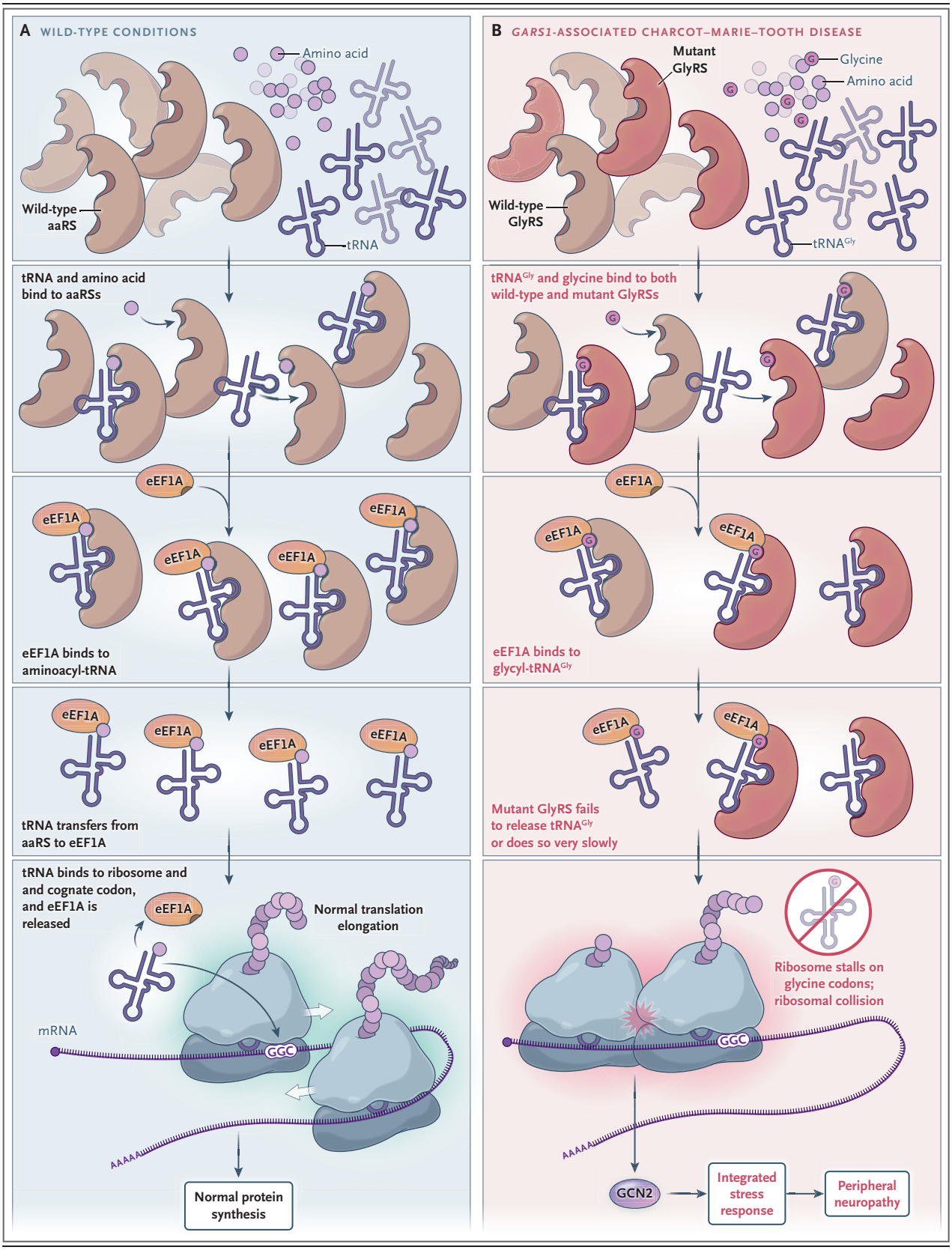


Figure 3 (facing page). Molecular Pathogenesis of Mutant tRNA Synthetase–Associated Charcot–Marie–Tooth Disease.

As shown in Panel A, in wild-type conditions, aminoacyl-tRNA synthetases (aaRSs) bind their cognate tRNA and amino acid and catalyze aminoacylation (Panel A). The charged tRNA is transferred to eEF1A, which delivers the tRNA to the ribosome during translation elongation. As shown in Panel B, in *GARS1*-associated Charcot–Marie–Tooth disease, both wild-type and mutant versions of glycyl-tRNA synthetase (GlyRS) are present. Charcot–Marie–Tooth disease–associated mutant GlyRSs bind $tRNA^{Gly}$ and may or may not aminoacylate but fail to release $tRNA^{Gly}$ or release it at a very slow pace. This reduces the cellular pool of $tRNA^{Gly}$ below a critical threshold, leading to insufficient $tRNA^{Gly}$ available for aminoacylation by the wild-type GlyRS, an insufficient supply of glycyl-$tRNA^{Gly}$ to the ribosome, and ribosome stalling on glycine codons. Stalled ribosomes and the ensuing ribosome collisions induce GCN2-mediated activation of the integrated stress response, which elicits peripheral neuropathy.

number of neurodegenerative diseases. In many diseases, activation of the stress response may promote neurodegeneration, but in some diseases (e.g., CMT1B), activation appears to be protective. Further investigation is warranted to identify the mechanism or mechanisms underlying these diverse effects of stress response activation on neurodegeneration. The effects may be cell type–specific. Indeed, in most neurodegenerative diseases, non-neuronal cell types (e.g., microglia, astrocytes, and oligodendrocytes) have been shown to contribute to neurodegeneration through non–cell-autonomous mechanisms (Table 1). For example, cell type–specific effects may explain why activation of the stress response appears to be beneficial in CMT1B, a demyelinating form of Charcot–Marie–Tooth disease, but detrimental in Charcot–Marie–Tooth disease type 2D, an axonal form of the disease. The integrated stress response is activated in myelinating Schwann cells in CMT1B but is activated in motor and sensory neurons in Charcot–Marie–Tooth disease type 2D. Currently, it is often unclear in which cell types stress response activation, translation defects, or both occur, and the contribution of aberrant translation to neurodegeneration in the relevant cell types is also unclear. Future research should investigate the degree to which translation defects causally contribute to neurodegeneration and whether translation defects may help to explain the cell type specificity of neurodegenerative diseases and the age at symptom onset.

Another outstanding question is why the brain is particularly sensitive to translation defects. One possibility is that neurons are typically large, highly polarized, postmitotic cells with long processes that make up most of the cellular volume, and these cells have high metabolic activity. These characteristics may sensitize neurons to prolonged metabolic stress. Current and future insights should pave the way for targeting translation as a therapeutic approach to neurodegenerative diseases. For instance, increasing $tRNA^{Gly}$ levels may constitute a novel therapeutic approach for GlyRS-associated Charcot–Marie–Tooth disease.[75] Clinical trials of an ISRIB derivative[95] and an eIF2B activator[96] for the treatment of ALS are under way. It is anticipated that inhibitors of the eIF2 kinases will be evaluated in clinical trials for the treatment of neurodegenerative diseases in the coming years. Furthermore, therapeutic approaches aimed at reducing the production of neurodegenerative disease–associated proteins should mitigate their aberrant interaction with the mRNA translation machinery and the ensuing mRNA translation defects. Finally, it is anticipated that additional genes encoding mRNA translation components will be linked to rare genetic forms of neurodegenerative diseases in the future, expanding our understanding of mRNA translation defects as a pathogenic mechanism in neurodegenerative diseases.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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