Published in final edited form as:

Adv Neurobiol. 2018 ; 20: 283–301. doi:10.1007/978-3-319-89689-2_11.

Lost in Translation: Evidence for Protein Synthesis Deficits in ALS/FTD and Related Neurodegenerative Diseases

Erik M. Lehmkuhl and

Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ, USA

Daniela C. Zarnescu

Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ, USA,
Department of Neuroscience, University of Arizona, Tucson, AZ, USA, Department of Neurology,
University of Arizona, Tucson, AZ, USA

Abstract

Cells utilize a complex network of proteins to regulate translation, involving post-transcriptional processing of RNA and assembly of the ribosomal unit. Although the complexity provides robust regulation of proteostasis, it also offers several opportunities for translational dysregulation, as has been observed in many neurodegenerative disorders. Defective mRNA localization, mRNA sequestration, inhibited ribogenesis, mutant tRNA synthetases, and translation of hexanucleotide expansions have all been associated with neurodegenerative disease. Here, we review dysregulation of translation in the context of age-related neurodegeneration and discuss novel methods to interrogate translation. This review primarily focuses on amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), a spectrum disorder heavily associated with RNA metabolism, while also analyzing translational inhibition in the context of related neurodegenerative disorders such as Alzheimer's disease and Huntington's disease and the translation-related pathomechanisms common in neurodegenerative disease.

Keywords

mRNA; Translation; Ribosome; ALS; FTD; TDP-43; c9orf72; RNA-binding proteins

11.1 Introduction

In eukaryotes, normal translation relies on the assembly of a small (40S) and a large (60S) ribosomal subunit into fully assembled (80S) ribosomes. Each subunit comprises several ribosomal proteins and RNAs (rRNAs) that work together to catalyze protein synthesis using messenger RNA (mRNA) as a template (Fig. 11.1a). Translation is a major contributor to protein homeostasis (proteostasis) and its dysfunction has the potential to affect all cellular functions [1].

In addition to canonical AUG-dependent translation, a non-AUG version, known as Internal Ribosome Entry Site (IRES)-mediated translation, can take place via ribosomal attachment

and elongation independent of a start codon (Fig. 11.1bi). Although no consensus sequence is known, the majority of IRESs are located near the 5' end of the mRNA [2]. Interestingly, tau, one of the major proteins implicated in Alzheimer's disease, has been shown to undergo IRES translation, although the contribution, if any, of this mechanism to pathogenesis remains unknown [3]. A version of IRES translation is Repeat Associated Non-AUG (RAN) translation (Fig. 11.1bii), which has been associated with mutant microsatellite expansions in traditionally noncoding portions of the genome such as untranslated regions (UTRs) or introns, and more recently with coding regions (recently reviewed in [4, 5]). In the absence of an AUG codon, translation is possible because the hairpin structure formed by microsatellite mRNA can mimic the methionine tRNA that initiates translation [6]. Although dipeptide repeats generated via RAN translation and their distinct contribution to disease will be briefly discussed in this chapter, a more indepth review has recently been published [7].

While the majority of translation occurs within the cytosol or ER bound ribosomes, 13 vital components of the oxidative phosphorylation complex are translated within the mitochondria, which host their own translational machinery including different ribosomal subunits, initiation factors, and tRNAs [8].

Regardless of its type, the complexity of translation as a highly regulated stepwise process provides numerous opportunities for errors caused by inhibition or deficits at any of these stages (see Fig. 11.1). Translation dysregulation has been implicated in several hereditary neurological disorders (reviewed in [9]). For example, loss-of-function mutations in one of five genes encoding subunits for the eukaryotic translation initiation factor eIF2B result in childhood ataxia, characterized by infant encephalopathy and later onset cognitive and motor impairment [10]. In Charcot-Marie-Tooth disease, an inherited neurodegenerative disorder, mutations in glycyl-tRNA synthetase (*GARS*), one of five tRNA synthetases linked to disease, disrupts translation [11]. FMRP, a protein implicated in Fragile X syndrome, regulates translation, in part by associating with ribosomes via direct binding to L5 protein [12]. Furthermore, mutations in *RPS19*, a small ribosomal subunit, have been associated with Blackfan Diamond Anemia, a deficiency in red blood cells that also leads to cognitive dysfunction [13]. Deficient mitochondrial translation has also been implicated in several neurological disorders; non-functional mitochondrial aspartyl or glutamyl-tRNA synthetases lead to leukoencephalopathy associated with ataxia, spasticity, and cognitive decline [9, 14].

Additionally, inhibition of translation can contribute to age-related neurodegenerative disorders. For example, in amyotrophic lateral sclerosis (ALS), TDP-43 sequesters mRNA away from translating ribosomes [15]. In Alzheimer's disease, microRNA-29, which regulates the expression of memory associated mRNAs (e.g., *BACE1*, a secretase implicated in the formation of pathogenic amyloid plaques) is downregulated causing uptranslation of its targets [16]. Translation is also expected to be affected by nucleolar stress and reduced rRNA biogenesis that have recently been associated with hexanucleotide repeat expansion (G4C2 HRE) within the first intron of *c9orf72*, the most common cause of ALS/FTD [17]. The role of translation in progressive neurodegeneration disorders is an exciting, developing field that is poised to uncover new therapeutic strategies. This chapter will focus on the

mechanisms by which errors in translation contribute to age-related neurodegenerative disorders with references to modern methodologies for probing translation deficits in vivo.

11.2 Translational Alterations in Different Types of ALS and ALS/FTD

ALS is a progressive neurodegenerative disorder causing death of motor neurons [18]. Although 90% of ALS cases are sporadic, several genetic loci have been linked to both familial and sporadic cases and have been shown to be involved in a plethora of biological process ranging from ribostasis (e.g., *TARDP*, *FUS*, *senataxin*, *Gle1*) to proteostasis (e.g., *ubiquilin*, *SOD1*, *VCP*) [19]. Although these different gene products contribute directly to various specific aspects of cellular function, translation has been found to be directly or indirectly altered in the context of disease causing mutations, or in the context of wild-type TDP-43 pathology, which represents 97% of ALS and 45% of FTD cases. Recently, several genes linked to ALS were also shown to cause FTD leading to reframing of ALS and FTD as a spectrum disorder [20]. Here, we summarize the current state of the field in regards to translation dysregulation in different types of ALS/FTD.

SOD1—

SOD1 (Superoxide Dismutase) was the first gene associated with ALS in 1993 and remains solely associated with motor neuron disease across the ALS/FTD continuum. In a landmark paper, Bruijn et al. demonstrated that a gain of toxic function rather than loss of enzymatic activity is responsible for neurodegeneration [21]. Although to date, the pathogenic mechanism of SOD1 has remained elusive, a great deal of evidence provides support for oxidative stress caused by mitochondrial dysfunction. Several hypotheses exist to explain the mechanism by which mutant SOD1 affects mitochondrion dysfunction (reviewed in [22]). Among these, Tan et al. showed that in *SOD1^{G93A}* ALS mice, mutant SOD1 induces a conformational change in Bcl-2, which leads to reduced permeability of the mitochondrial membrane through altered interactions with Voltage Dependent Anion Channel 1 [23]. The loss of mitochondrial membrane polarity and subsequent oxidative stress lead to an increase in protein misfolding, causing a cascade of secondary effects including an unfolded protein response, which in turn inhibits global translation (reviewed in [24]).

Recently, Gal et al. discovered a novel role for mutant SOD1 pathogenesis [25]. In both *SOD1^{G93A}* mice and patient-derived fibroblasts, mutated SOD1 was identified in inclusions containing TIA1 and G3BP1, two core components of stress granules [26]. Additional disease-associated variants *SOD1^{A4V}* and *SOD1^{G85R}* co-precipitated with G3BP1, indicating that stress granule interaction affects the pathogenesis of multiple SOD1 mutants. Co-precipitation occurs even following RNase treatment suggesting that the G3BP1-mutant SOD1 interaction is not RNA dependent. Indeed, co-precipitation of truncated G3BP1 mutants and *SOD1^{A4V}* indicated that the RNA Recognition Motif (RRM) of G3BP1 is necessary and sufficient for the G3BP1-*SOD1^{A4V}* binding. Most importantly, the presence of *SOD1^{A4V}* negatively correlated with stress granule formation, indicating a causal relationship between mutant SOD1-G3BP1 binding and stress granule dynamics [25]. Although the mechanism remains unclear, this work establishes a novel relationship between SOD1 mutants and stress granule dynamics, and suggests alterations in translation.

This possibility was addressed in a 2015 study, which examined translational changes that occur in mouse motor neurons, astrocytes, and oligodendrocytes during SOD1 driven ALS pathogenesis [27]. Previous cell-type-specific studies have relied on physical separation of the cell type of interest; this has numerous limitations including potential contamination and exclusion of axons and dendrites. This innovative study employed tagged ribosome affinity purification (TRAP), which allows the identification of cell-specific translomes from intact, whole organisms (Fig. 11.2).

To define cell-type-specific translomes, Sun et al. expressed GFP-RPL10 in motor neurons, astrocytes, and oligodendrocytes of *SOD1*^{G37R} mice using cell-specific promoters *Chat*, *Aldh1l1*, and *Cnp1*, respectively [27]. Importantly, the *SOD1*^{G37R} mutant line recapitulated the expression levels of endogenous SOD1, which is expressed in astrocytes and oligodendrocytes at 30% and 40% of motor neuron levels, respectively. The spinal cords of the mice were isolated at 8 months of age, corresponding to disease onset when muscle denervation has begun but phenotypes are not overtly present. Immunoprecipitation of GFP-RPL10 and subsequent RNA-seq followed by bioinformatics defined the translome of each cell type.

At 8 months, Sun et al. observed that motor neurons exhibit upregulated translation of the components of the PERK (PRKR-like ER kinase)-mediated unfolded protein response (UPR) [27]. Protein misfolding induces PERK to phosphorylate eukaryotic initiation factor 2 alpha (eIF2α), which in turn upregulates translation of ATF4, a transcription factor that enhances the UPR response [28]. Translation of both ATF4 and its target transcripts (e.g., heat shock proteins *HSF1* and *HSF2*) was increased. Interestingly, the other components of UPR, namely ATF6 and IRE1, were not induced in disease onset motor neurons.

Laser microdissection was used to isolate motor neurons at the early symptomatic age of 10.5 months. Quantitative PCR experiments showed elevated ATF4 expression indicating that the UPR response continues through disease progression and is not limited to onset. A parallel experiment using *SOD1*^{G85R} mice concluded that PERK-mediated UPR was also upregulated in these mutants, consistent with the idea that ER stress is common to SOD1 mediated pathogenesis.

Using the TRAP approach, 8 months old mouse astrocytes revealed an upregulation of inflammation related proteins (e.g., transcription factors *Cedpb* and *Cedpd*) while mRNAs encoding transcriptional co-activators for metabolic genes (e.g., *PRRX1* and *SERTAD2*) experienced decreased translation. Upregulation of inflammation is characteristic of an astrocyte response to neuron damage. However, the increased translation of transcription factor *PGC1α*, related to metabolism and nuclear receptors, was also observed. Since upregulation of *PGC1α* is not characteristic of astrogliosis, it suggests that at least in part, *SOD1*^{G37R} pathogenesis in astrocytes is independent of neuronal damage and may reflect a cell autonomous response to mutations in *SOD1* by astrocytes.

In contrast, oligodendrocytes exhibited minimal translational changes at 8 months, when mice were presymptomatic. However, profiling their translation again at the early symptomatic age of 10.5 months revealed that oligodendrocytes exhibit increased translation

of transcripts involved in phagocytosis (e.g., *Rac2* and *Phosphoinositide Phospholipase C*) accompanied by a predicted decrease in proteins involved in myelination (e.g., CAMK2 β).

From their findings, Sun et al. propose a model of SOD1-mediated pathogenesis where mutant SOD1 first induces motor neuron damage through the induction of ER stress [27]. Motor neurons may be selectively vulnerable because of high SOD1 expression and low ER chaperone presence. The effects of motor neuron damage are then amplified by subsequent damage to astrocytes and oligodendrocytes. The translational profiling conducted by Sun et al. provides elegant insights into translational alterations in vivo, in a cell type and temporal-specific fashion, that highlight the central role of motor neurons in disease.

TDP-43—

Encoded by the TAR DNA-Binding (*TARDP*) gene, TDP-43 is an RNA-binding protein comprising two RRM domains [29]. Remarkably, >97% of patients, regardless of etiology (with a couple of exceptions, including *SOD1* and *FUS* mutations) exhibit proteinaceous aggregates containing the RNA-binding protein TDP-43 [30]. TDP-43 has been implicated in several aspects of RNA processing including mRNA transport and localization to the distal ends of neurites including synapses [31–33]. While TDP-43 is normally required for RNA processing (e.g., splicing) [29], RNA binding is also required for toxicity [34], highlighting the involvement of RNA-based mechanisms in TDP-43 pathogenesis.

Recent studies have shed light into the mechanism by which TDP-43 contributes to ALS pathogenesis. In 2014, Coyne et al. [32] used a previously described *Drosophila* model of ALS [35, 36] based on TDP-43 overexpression to identify *futsch* as physiologically relevant target of TDP-43 regulation. *Futsch* mRNA was shown to be increased in motor neuron cell bodies, but decreased at neuromuscular synapses, consistent with failed mRNA localization. Polysome fractionations of ALS larvae indicated a shift of *futsch* mRNA from actively translating ribosomes to untranslated ribonucleoprotein particle (RNP) fractions consistent with translation inhibition [32]. This combination of defects in RNA localization and translation leads to increased levels of Futsch protein in motor neuron cell bodies, which was confirmed to also occur for its mammalian homolog, MAP1B, in spinal cords from ALS patients. This pathological alteration in Futsch/MAP1B, a microtubule stabilizing protein is consistent with neuromuscular junction (NMJ) instability, which was observed in the fly model. Notably, restoration of *futsch* levels by genetic overexpression mitigates ALS phenotypes including locomotor defects, TDP-43 aggregation, and reduced lifespan suggesting that *futsch* is an important mediator of TDP-43 toxicity in vivo.

TDP-43 knock-down studies in mouse hippocampal neurons have indicated that *Rac1* levels increase at the translational level and this affects spine morphogenesis in dendrites [37]. Together with observations that AMPAR clustering is increased following synaptic stimulation, these findings support a role for TDP-43 in plasticity. The human disease relevance of these observations remains to be established in future studies.

Recently, an interesting mechanistic connection has been identified between ribostasis and proteostasis [15]. Using the same *Drosophila* model of ALS [35, 36] the authors identified *hsc70-4* mRNA as a candidate target of mutant but not wild-type TDP-43 [15]. Hsc70-4 is a

conserved member of the Hsc70 family of constitutive chaperones with several roles in protein folding, degradation and various cellular processes including stress response, and chaperone-mediated autophagy [38]. Specifically, Hsc70-4 regulates synaptic vesicle cycling, and just like its cognate mRNA was found to associate preferentially with mutant TDP-43. The consequence of this preferential association with mutant TDP-43 is the sequestration of *hsc70-4* mRNA accompanied by translation inhibition, which in turn leads to defects in the synaptic vesicle endocytosis. A similar post-transcriptional reduction was observed in C9 ALS fly and patient-derived motor neurons, although it remains to be determined whether this is caused by translation inhibition as was the case with TDP-43 models. Notably, restoration of Hsc70-4 through genetic overexpression mitigated ALS phenotypes in a variant-dependent manner suggesting that although both wild-type and mutant TDP-43 contribute to ALS pathogenesis, they do so through distinct mechanisms [15].

Additional links between TDP-43 and protein synthesis have been uncovered by biochemical studies showing its association with several RNA-binding proteins involved in translation including eukaryotic initiation factors and Fragile X Mental Retardation Protein (FMRP) [39–43]. FMRP overexpression was found to attenuate locomotor dysfunction and increase lifespan in a fly model of ALS based on TDP-43 [42]. Genetic interactions and fractionation experiments collectively led to a model whereby FMRP remodels TDP-43/RNA complexes and releases sequestered mRNA, which can subsequently be translated and mitigate TDP-43 toxicity. Interestingly, FMRP and TDP-43 appear to share translation targets including *Rac1* and *futsch* mRNAs, highlighting previously unknown common mechanisms between neurodevelopmental conditions such as Fragile X syndrome and neurodegenerative diseases like ALS/FTD.

TDP-43 has also been found to regulate translation globally [44]. Using an Affymetrix exon array, Fiesel et al. [44] evaluated splicing variants in HEK293E human embryonic kidney cells following knockdown of TDP-43 with small interfering RNA (siRNA). This study showed that loss of TDP-43 induced alternative splicing of *S6 kinase 1 Aly/REF-like target* (*SKAR*). In addition to being previously associated with spliced mRNA, SKAR also recruits S6 Kinase 1 to protein-mRNA granules to promote translation downstream of mTOR signaling [45]. Knock-down of TDP-43 causes exon 3 exclusion and generation of SKAR β , which results in increased phosphorylation of S6 K1 and its targets, leading to increased global translation [44]. It remains to be determined whether global translation is also altered in patients. The most compelling evidence so far of global translation dysregulation comes from findings that genetic and pharmacological inhibition of eIF2 α phosphorylation mitigates ALS phenotypes in fly and cultured cells models [46]. However, given the intimate connections between UPR and global translation that eIF2 α mediates, more studies are needed to determine the extent of translation dysregulation in disease pathogenesis.

Given its known interactions with protein partners, TDP-43 appears to be involved in the regulation of translation at multiple steps. Studies have identified both a normal role for TDP-43 in the regulation of global translation in cultured cells, and specific mRNAs targets, including mutant-specific targets in motor neurons, in the context of disease. A distinction needs to be made between TDP-43's normal role in various cell types and how that role

changes in disease and more studies are needed to address this important question. The variety of ways in which TDP-43 interacts with the translational machinery leads to additional questions regarding the role of TDP-43 and RNA in ALS pathogenesis.

FUS—

Mutations in *Fused in Sarcoma* (FUS), which encodes a nuclear RNA-binding protein, have also been associated with 4% of familial ALS with autopsy showing cytoplasmic inclusions of FUS [47]. Although no specific alterations have been identified in translation in the context of FUS ALS, given the aggregation of mutant FUS^{P525L} in complexes containing nuclear-cytoplasmic shuttling proteins (e.g., hnRNP A1 and A2), spliceosome assembling proteins (e.g., SMN1), and mRNA [48], it is reasonable to predict indirect changes in the transcriptome caused by aberrant protein and protein-RNA interactions.

c9orf72—

Hexanucleotide repeat expansions (HRE) in *c9orf72* have been recently identified as the most common cause of familial ALS [49, 50]. These G4C2 repeats lie within the first intron of *c9orf72* and range from 2 to 10 in the normal population, and 90 to several hundreds in disease. Repeat expansions as low as 20 have been identified in ALS cases, but a causal relationship has not been established between the size of expansions and disease, and evidence for multiple gene mutation contributions has been found in carrier families [51]. Much research has focused on discerning the normal function of *c9orf72*, a putative DENN protein [52], and the contribution of hexanucleotide repeat expansions to disease. Although evidence exists to support several disease mechanisms including haploinsufficiency, RNA foci, and dipeptide repeat (DPR)-mediated toxicity, the specific pathomechanism of *c9orf72* remains unclear and subject to controversy [53]. A most remarkable discovery made in regards to *c9orf72* pathomechanism is the finding of nuclear pore alterations and defects in nucleo-cytoplasmic shuttling [17, 54, 55]. Accompanying these phenotypes are defects in RNA SG assembly, translation, and ribosome biogenesis, discussed later.

RNA foci and toxicity—

Overexpression of G4C2 HREs of various lengths led to reduced transcription of stress granule proteins TIA-1 and HuR indicating a role for RNA foci in stress granule assembly [56]. However, because these HREs also generated DPRs, the contribution of the latter cannot be excluded. Using elegant live imaging approaches, Schweizer Burguete et al. observed that *c9orf72* HREs foci colocalize with FMRP and translocate bi-directionally within neurites. Interestingly, the presence of HRE increased protein levels for both FMRP and PSD-95, a protein whose translation is facilitated by FMRP, indicating that the *c9orf72* HRE may alter local protein translation [57]. These studies propose that the HREs induce neurodegenerative phenotypes by altering mRNA localization to synapses, a phenotype previously associated with other types of ALS [15, 32, 33].

RAN translation—

As mentioned above, RAN (Repeat Associated Non-AUG translation) is a version of IRES-based translation mechanism that causes the repeat expansions to be translated into dipeptide

repeats (DPRs). In *c9orf72*-mediated ALS, RAN translation of the G4C2 repeat and its anti-sense mRNA result in poly(GA), poly(GP), poly(GR), poly(PA), and poly(PR) dipeptides, all of which have been detected in patient tissues. One study implicated poly(GA) dipeptides as the primary aggregate inducing DPR [58], however another study concluded that expression of poly(GR) and poly(PR) induced neurodegenerative phenotypes while the three dipeptide products lacking arginine did not [59]. Although which DPRs are toxic remains an actively investigated question in the field, their effect on translation is undisputed.

In the context of ALS, DPRs have been associated with the disruption of translation and nuclear-cytoplasmic transport. Using Surface Sensing of Translation (SUnSET) [60] (Fig. 11.3), Kanekura et al. observed that poly(PR) and poly(GR) DPRs inhibit global translation in NSC34 motor neuron like cells [61]. The arginine-containing DPRs were found to form aggregates containing RNA-binding proteins (e.g., FUS and TDP-43) along with RNA. The model emerging from this study is that the hydrophobic DPR aggregates block translation by preventing initiation factors from interacting with mRNA.

Poly(PR) and poly(GR) DPRs have also been implicated in post-transcriptional processing, nuclear cytoplasmic transport, and rRNA biogenesis, which can ultimately affect translation. Jovicic et al. also identified several genes related to rRNA biogenesis (e.g., *efg1* and *nsr1*) as significant modifiers of poly(PR) toxicity [17]. Additionally, poly(PR) and poly(GR) peptides colocalize with nucleoli, the site of rRNA synthesis [62]. Although the dysregulation of ribogenesis has been associated with *c9orf72* HRE in multiple studies, the mechanism remains poorly understood. Overall, although the role of *c9orf72* in both healthy and neurodegenerative individuals has been heavily studied in recent years, significant work remains to be done regarding the molecular mechanism and to precisely determine the contributions of G4C2 expanded RNA or the translated dipeptide products to disease. The mixed spectrum of results to date may reflect heterogeneous responses to HREs and DPRs among different cell types in the nervous system (Fig. 11.4).

11.3 RAN Translation Beyond ALS/FTD

The first reports of RAN translation were made in association with CAG expanded transcripts in *SCA8* and Muscular Dystrophy type I (DM1) [6]. DM1 is caused by CTG repeat expansions within the myotonic dystrophy protein kinase (*DMPK*). Once transcribed, the expanded CUG mRNA forms a double-stranded structure that sequesters muscleblind (MBNL1), an RNA-binding protein involved in splicing [63]. As a result, CUGBP1 is upregulated, which together with MBNL1 sequestration leads to defects in the fetal to adult splicing transition and disease pathogenesis. Recent studies, however, have proposed an additional pathomechanism whereby the repeat expansions undergo RAN translation [6], albeit the mechanism by which RAN products contribute to disease is unknown. A recent review on this topic provides an excellent overview of the increasingly complex mechanisms behind myotonic dystrophy [64].

Since the initial discovery of RAN translation, additional microsatellite expansion disorders including Fragile X Tremors Ataxia Syndrome (FXTAS) and Huntingtin's disease have been added to the list of conditions in which bidirectional expanded transcripts produce RAN

proteins [4, 5]. These novel and unexpected peptides contribute to toxicity challenging existing paradigms about disease mechanisms wherever they are found.

Huntington's disease (HD)—

HD is an autosomal dominant neurodegenerative disorder [65]. The expansion of a CAG repeat region within the coding region of the huntingtin gene (*HTT*) leads to disease onset between the ages of 30 and 50 and causes progressive loss of neuron function [65]. The protein product of *HTT*, Huntingtin, is associated with microtubule-based trafficking of vesicles and mRNAs within neurons [66]. Initial suggestions that protein synthesis may be altered in HD came from fibroblasts showing that in cells cultured from Huntington's patients, RNA accumulates in the nucleus and is not properly translated [67]. A more recent report shows that *HTT* repeat expansions also undergo RAN translation that can drive neurodegeneration through the dysregulation of nuclear-cytoplasmic transport [68]. Similar phenotypes including nuclear envelope morphology, pore architecture, and RNA export defects were found in a parallel study, although no RAN translation products were reported [69]. The discovery of RAN translation by Grima et al. [68] led to proposing a mechanism whereby its products cause these newly discovered phenotypes by specifically altering nuclear pores and inhibiting RANGAP1, a GTPase-activating protein necessary for nuclear cytoplasmic shuttling. Further substantiating this model is the fact that RANGAP1 overexpression or pharmacological restoration of nuclear transport rescued HD phenotypes across multiple model system [68].

Fragile X tremor ataxia syndrome (FXTAS)—

FXTAS is caused by the expansion of CGG repeats in the 5' UTR of the Fragile X Mental Retardation gene 1 (*FMR1*) [70–72]. The length of the CGG expansions determines the phenotypic outcome, with repeats >200 causing complete loss of transcription and absence of FMRP, while intermediate length repeats (50–200) lead to increased transcript but reduced protein production [73]. The intermediate expansion was associated with intention tremors, ataxia, dementia, and parkinsonism occurring in aging individuals [74]. Mechanistically, CGG repeats were shown to sequester RNA-binding proteins thereby dysregulating their activity within cells [75, 76]. An example relevant to translation regulation is DGCR8, a miRNA processing factor, which binds to expanded *FMR1* mRNA; this leads to decreased levels of mature microRNAs, which in turn can impact the translome by impairing the translation of their mRNA targets [77]. Several recent studies identify RAN translation products, specifically polyG peptides produced from CGG expanded repeats using an ACG codon as start [78–80]. Elegant experiments in mouse models and patient-derived cells demonstrate that RAN translation-derived polyG peptides but not CGG RNA alone are responsible for FXTAS phenotypes [80].

11.4 Translation Dysregulation in Alzheimer's Disease

Recent studies have associated dysregulation of protein-mRNA complexes with Alzheimer's disease pathology [81]. Tau is a microtubule-associated protein whose aggregation and hyper-phosphorylation is a hallmark of Alzheimer's with pathology predicted to be driven in part by failed axonal transport [82]. Recent studies identified pathological tau in complex

with TIA1 [83], a core component of stress granules. Interestingly, tau-TIA1 binding was found to have a positive correlation with stress granule formation suggesting possible consequences on translation that will have to be elucidated in future studies. Additionally, TIA1 mediates translational inhibition of many stress response genes including P53, a major regulator of DNA damage repair [84]. It remains to be determined if susceptibility to, or DNA damage itself, may be involved in AD pathogenesis. Additionally, a dichotomous relationship was recently observed between TDP-43 and tau levels during Alzheimer's pathogenesis, with TDP-43 being shown to regulate tau protein expression by destabilizing its cognate mRNA [85].

Translation efficiency was also shown to be deficient in Alzheimer's brains and was noted to be an early event in disease. Ding et al. showed that in patient brain extracts, although the same quantity of polyribosome material was produced in control and disease cases, the translational efficiency of the polyribosomes was reduced by >60% in the inferior parietal lobe (IP) and superior middle temporal gyri (SMTG), albeit no significant reduction was observed in the cerebellum [86]. Regarding the mechanism of translation deficiency, tRNA^{Asn} and 5S rRNA were significantly reduced in the IP of Alzheimer's patients together with increased oxidation of 28S rRNA. In contrast, the cerebellum of Alzheimer's patients exhibited increased phosphorylation of eIF2 α and p70S6 [86]. While the former is associated with increased unfolded protein response and stress granule formation, the latter is associated with activation of the mTOR pathway and increased translation. Collectively, these findings provide intriguing links between Alzheimer's disease pathogenesis and translation through stress granules, initiation factors, and rRNA; however, the precise involvement of translation in Alzheimer's remains unknown.

11.5 Micro RNAs (miRNAs) and Translation Regulation

miRNAs are noncoding RNAs that can control gene expression by inhibiting mRNA translation or by selective degradation of transcripts (reviewed in [87]). It has been shown that TDP-43 aggregates sequester Dicer and DROSHA, two key RNA-binding proteins required for generating functional miRNA, thus implicating miRNA maturation in ALS pathogenesis [88]. DROSHA was also shown to form aggregates with RAN translation derived DPRs in patient tissues [89]. Additionally, XP05, which is required for precursor miRNA export from the nucleus, was identified as modifier of *c9orf72* HRE and TDP-43-based pathogenesis among other nuclear-cytoplasmic transport proteins [17, 54, 90]. Consequent reduced Dicer and DROSHA activity, and inhibited nuclear cytoplasmic transport potentially explain decreased global miRNA levels observed in multiple forms of ALS (recently reviewed in [91]). Several miRNAs required for synaptic development and maintenance including *miR-9* and *miR-124* were found to be altered in ALS/FTD patient derived cells and tissues suggesting the possibility that specific miRNAs may mediate aspects of toxicity in disease [92, 93]. Although the precise role of miRNAs in disease remains unclear, existing evidence supports the possibility of both a global and target-specific inhibition of miRNA synthesis as a contributor to ALS/FTD pathogenesis (recently reviewed in [91, 94]).

11.6 Concluding Remarks

Recent studies have provided compelling evidence that ALS/FTD and even Alzheimer's disease exhibit defects in multiple steps of RNA processing including protein synthesis. Several neurodegeneration-associated proteins are involved in mRNA export, trafficking, localization, and translation. These processes offer a plausible explanation for the unique pathogenesis observed in neurons, which have dendritic and axonal extremities requiring local translation and mRNA transport across distances vastly larger than the cell body (recently reviewed in [95–97]). The recent discovery that certain ribosomal subunits preferentially translate subsets of mRNA [98] provides an additional layer to the complex mosaic that is translation within neurons.

Evidence exists to support both global and target-specific dysregulation of translation. Globally, activation of the PERK pathway including phosphorylation of eIF2 α inhibits global translation at the initiation step by inhibiting the incorporation of eIF2 α into ribosomal complexes [99]. The inhibition of PERK has provided encouraging results in attenuating neurodegenerative phenotypes as it mitigated TDP-43-dependent phenotypes in flies and cultured mouse cells [46], and it restored synaptic protein levels and motor function in models of prion-mediated neurodegeneration [100]. Given findings that PERK is also upregulated in SOD1 mice [27], this approach could be extended to additional ALS types. However, pharmacological inhibition of translation did not rescue memory defects in mouse models of Alzheimer's disease [101, 102].

The evidence for specific mRNA targets further substantiates existing hypotheses that axonal transport, neuronal cytoskeleton, and synaptic vesicle cycling are underlying the synaptopathy associated with neurodegeneration. While these provide more specificity to any future interventions, it remains difficult to prioritize which mRNA target carries more physiological significance, and targeting multiple targets simultaneously poses significant challenges. Clearly, Aristotle's famous quote "The more you know, the more you know you don't know" remains relevant today; a lot is yet to be learned about the intricacies of translation dysregulation in neurodegeneration, specific RNA targets and processes, from disease onset to motor neuron failure and death.

References

1. Aitken CE, Lorsch JR. A mechanistic overview of translation initiation in eukaryotes. *Nat Struct Mol Biol.* 2012;19(6):568–76. [PubMed: 22664984]
2. Komar AA, Hatzoglou M. Cellular IRES-mediated translation: the war of ITAFs in patho-physiological states. *Cell Cycle.* 2011;10(2):229–40. [PubMed: 21220943]
3. Veo BL, Krushel LA. Translation initiation of the human tau mRNA through an internal ribosomal entry site. *J Alzheimers Dis.* 2009;16(2):271–5. [PubMed: 19221416]
4. Cleary JD, Ranum LP. New developments in RAN translation: insights from multiple diseases. *Curr Opin Genet Dev.* 2017;44:125–34. [PubMed: 28365506]
5. Gao FB, Richter JD, Cleveland DW. Rethinking unconventional translation in neurodegeneration. *Cell.* 2017;171(5):994–1000. [PubMed: 29149615]
6. Zu T, Gibbens B, Doty NS, Gomes-Pereira M, Huguet A, Stone MD, et al. Non-ATG-initiated translation directed by microsatellite expansions. *Proc Natl Acad Sci U S A.* 2011;108(1):260–5. [PubMed: 21173221]

7. Freibaum BD, Taylor JP. The role of dipeptide repeats in C9ORF72-related ALS-FTD. *Front Mol Neurosci.* 2017;10:35. [PubMed: 28243191]
8. Boczonadi V, Horvath R. Mitochondria: impaired mitochondrial translation in human disease. *Int J Biochem Cell Biol.* 2014;48:77–84. [PubMed: 24412566]
9. Scheper GC, van der Knaap MS, Proud CG. Translation matters: protein synthesis defects in inherited disease. *Nat Rev Genet.* 2007;8(9):711–23. [PubMed: 17680008]
10. van der Knaap MS, Leegwater PA, Konst AA, Visser A, Naidu S, Oudejans CB, et al. Mutations in each of the five subunits of translation initiation factor eIF2B can cause leukoencephalopathy with vanishing white matter. *Ann Neurol.* 2002;51(2):264–70. [PubMed: 11835386]
11. Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin SQ, et al. Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. *Am J Hum Genet.* 2003;72(5):1293–9. [PubMed: 12690580]
12. Chen E, Sharma MR, Shi X, Agrawal RK, Joseph S. Fragile x mental retardation protein regulates translation by binding directly to the ribosome. *Mol Cell.* 2014;54(3):407–17. [PubMed: 24746697]
13. Kubik-Zahorodna A, Schuster B, Kanchev I, Sedlacek R. Neurological deficits of an Rps19(Arg67del) model of Diamond-Blackfan anaemia. *Folia Biol (Praha).* 2016;62(4):139–47. [PubMed: 27643579]
14. Steenweg ME, Ghezzi D, Haack T, Abbink TE, Martinelli D, van Berkel CG, et al. Leukoencephalopathy with thalamus and brainstem involvement and high lactate 'LTBL' caused by EARS2 mutations. *Brain.* 2012;135(Pt 5):1387–94. [PubMed: 22492562]
15. Coyne AN, Lorenzini I, Chou CC, Torvund M, Rogers RS, Starr A, et al. Post-transcriptional inhibition of Hsc70-4/HSPA8 expression leads to synaptic vesicle cycling defects in multiple models of ALS. *Cell Rep.* 2017;21(1):110–25. [PubMed: 28978466]
16. Hebert SS, Horre K, Nicolai L, Papadopoulou AS, Mandemakers W, Silahtaroglu AN, et al. Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. *Proc Natl Acad Sci U S A.* 2008;105(17):6415–20. [PubMed: 18434550]
17. Jovicic A, Mertens J, Boeynaems S, Bogaert E, Chai N, Yamada SB, et al. Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat Neurosci.* 2015;18(9):1226–9. [PubMed: 26308983]
18. Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat Rev Neurosci.* 2001;2(11):806–19. [PubMed: 11715057]
19. Schymick JC, Traynor BJ. Expanding the genetics of amyotrophic lateral sclerosis and frontotemporal dementia. *Alzheimers Res Ther.* 2012;4(4):30. [PubMed: 22835154]
20. Ling SC, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron.* 2013;79(3):416–38. [PubMed: 23931993]
21. Bruijn LI, Houseweart MK, Kato S, Anderson KL, Anderson SD, Ohama E, et al. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science.* 1998;281(5384):1851–4. [PubMed: 9743498]
22. Tafuri F, Ronchi D, Magri F, Comi GP, Corti S. SOD1 misplacing and mitochondrial dysfunction in amyotrophic lateral sclerosis pathogenesis. *Front Cell Neurosci.* 2015;9:336. [PubMed: 26379505]
23. Tan W, Naniche N, Bogush A, Pedrini S, Trotti D, Pasinelli P. Small peptides against the mutant SOD1/Bcl-2 toxic mitochondrial complex restore mitochondrial function and cell viability in mutant SOD1-mediated ALS. *J Neurosci.* 2013;33(28):11588–98. [PubMed: 23843527]
24. Hetz C, Mollereau B. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. *Nat Rev Neurosci.* 2014;15(4):233–49. [PubMed: 24619348]
25. Gal J, Kuang L, Barnett KR, Zhu BZ, Shissler SC, Korotkov KV, et al. ALS mutant SOD1 interacts with G3BP1 and affects stress granule dynamics. *Acta Neuropathol.* 2016;132(4):563–76. [PubMed: 27481264]
26. Kedersha N, Anderson P. Regulation of translation by stress granules and processing bodies. *Prog Mol Biol Transl Sci.* 2009;90:155–85. [PubMed: 20374741]

27. Sun S, Sun Y, Ling SC, Ferraiuolo L, McAlonis-Downes M, Zou Y, et al. Translational profiling identifies a cascade of damage initiated in motor neurons and spreading to glia in mutant SOD1-mediated ALS. *Proc Natl Acad Sci U S A*. 2015;112(50):E6993–7002. [PubMed: 26621731]
28. Harding HP, Calfon M, Urano F, Novoa I, Ron D. Transcriptional and translational control in the Mammalian unfolded protein response. *Annu Rev Cell Dev Biol*. 2002;18:575–99. [PubMed: 12142265]
29. Buratti E, Dork T, Zuccato E, Pagani F, Romano M, Baralle FE. Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. *EMBO J*. 2001;20(7):1774–84. [PubMed: 11285240]
30. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006;314(5796):130–3. [PubMed: 17023659]
31. Ishiguro A, Kimura N, Watanabe Y, Watanabe S, Ishihama A. TDP-43 binds and transports G-quadruplex-containing mRNAs into neurites for local translation. *Genes Cells*. 2016;21(5):466–81. [PubMed: 26915990]
32. Coyne AN, Siddegowda BB, Estes PS, Johannesmeyer J, Kovalik T, Daniel SG, et al. Futsch/ MAP1B mRNA is a translational target of TDP-43 and is neuroprotective in a Drosophila model of amyotrophic lateral sclerosis. *J Neurosci*. 2014;34(48):15962–74. [PubMed: 25429138]
33. Alami NH, Smith RB, Carrasco MA, Williams LA, Winborn CS, Han SS, et al. Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron*. 2014;81(3):536–43. [PubMed: 24507191]
34. Voigt A, Herholz D, Fiesel FC, Kaur K, Muller D, Karsten P, et al. TDP-43-mediated neuron loss in vivo requires RNA-binding activity. *PLoS One*. 2010;5(8):e12247. [PubMed: 20806063]
35. Estes PS, Boehringer A, Zwick R, Tang JE, Grigsby B, Zarnescu DC. Wild-type and A315T mutant TDP-43 exert differential neurotoxicity in a Drosophila model of ALS. *Hum Mol Genet*. 2011;20(12):2308–21. [PubMed: 21441568]
36. Estes PS, Daniel SG, McCallum AP, Boehringer AV, Sukhina AS, Zwick RA, et al. Motor neurons and glia exhibit specific individualized responses to TDP-43 expression in a Drosophila model of amyotrophic lateral sclerosis. *Dis Model Mech*. 2013;6(3):721–33. [PubMed: 23471911]
37. Majumder P, Chen YT, Bose JK, Wu CC, Cheng WC, Cheng SJ, et al. TDP-43 regulates the mammalian spinogenesis through translational repression of Rac1. *Acta Neuropathol*. 2012;124(2):231–45. [PubMed: 22760527]
38. Liu T, Daniels CK, Cao S. Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential. *Pharmacol Ther*. 2012;136(3):354–74. [PubMed: 22960394]
39. Sephton CF, Cenik C, Kucukural A, Dammer EB, Cenik B, Han Y, et al. Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. *J Biol Chem*. 2011;286(2):1204–15. [PubMed: 21051541]
40. Freibaum BD, Chitta RK, High AA, Taylor JP. Global analysis of TDP-43 interacting proteins reveals strong association with RNA splicing and translation machinery. *J Proteome Res*. 2010;9(2):1104–20. [PubMed: 20020773]
41. Majumder P, Chu JF, Chatterjee B, Swamy KB, Shen CJ. Co-regulation of mRNA translation by TDP-43 and Fragile X Syndrome protein FMRP. *Acta Neuropathol*. 2016;132(5):721–38. [PubMed: 27518042]
42. Coyne AN, Yamada SB, Siddegowda BB, Estes PS, Zaepfel BL, Johannesmeyer JS, et al. Fragile X protein mitigates TDP-43 toxicity by remodeling RNA granules and restoring translation. *Hum Mol Genet*. 2015;24(24):6886–98. [PubMed: 26385636]
43. Wang JW, Brent JR, Tomlinson A, Shneider NA, McCabe BD. The ALS-associated proteins FUS and TDP-43 function together to affect Drosophila locomotion and life span. *J Clin Invest*. 2011;121(10):4118–26. [PubMed: 21881207]
44. Fiesel FC, Weber SS, Supper J, Zell A, Kahle PJ. TDP-43 regulates global translational yield by splicing of exon junction complex component SKAR. *Nucleic Acids Res*. 2012;40(6):2668–82. [PubMed: 22121224]

45. Ma XM, Yoon SO, Richardson CJ, Julich K, Blenis J. SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. *Cell*. 2008;133(2):303–13. [PubMed: 18423201]
46. Kim HJ, Raphael AR, LaDow ES, McGurk L, Weber RA, Trojanowski JQ, et al. Therapeutic modulation of eIF2alpha phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models. *Nat Genet*. 2014;46(2):152–60. [PubMed: 24336168]
47. Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science*. 2009;323(5918):1208–11. [PubMed: 19251628]
48. Yamaguchi A, Takanashi K. FUS interacts with nuclear matrix-associated protein SAFB1 as well as Matrin3 to regulate splicing and ligand-mediated transcription. *Sci Rep*. 2016;6:35195. [PubMed: 27731383]
49. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron*. 2011;72(2):245–56. [PubMed: 21944778]
50. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*. 2011;72(2):257–68. [PubMed: 21944779]
51. van Blitterswijk M, van Es MA, Hennekam EA, Dooijes D, van Rheenen W, Medic J, et al. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. *Hum Mol Genet*. 2012;21(17):3776–84. [PubMed: 22645277]
52. Levine TP, Daniels RD, Gatta AT, Wong LH, Hayes MJ. The product of C9orf72, a gene strongly implicated in neurodegeneration, is structurally related to DENN Rab-GEFs. *Bioinformatics*. 2013;29(4):499–503. [PubMed: 23329412]
53. Gitler AD, Tsuiji H. There has been an awakening: emerging mechanisms of C9orf72 mutations in FTD/ALS. *Brain Res*. 2016;1647:19–29. [PubMed: 27059391]
54. Freibaum BD, Lu Y, Lopez-Gonzalez R, Kim NC, Almeida S, Lee KH, et al. GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature*. 2015;525(7567):129–33. [PubMed: 26308899]
55. Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, et al. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature*. 2015;525(7567):56–61. [PubMed: 26308891]
56. Maharjan N, Kunzli C, Buthey K, Saxena S. C9ORF72 regulates stress granule formation and its deficiency impairs stress granule assembly, hypersensitizing cells to stress. *Mol Neurobiol*. 2017;54(4):3062–77. [PubMed: 27037575]
57. Schweizer Burguete A, Almeida S, Gao FB, Kalb R, Akins MR, Bonini NM. GGGGCC microsatellite RNA is neuritically localized, induces branching defects, and perturbs transport granule function. *Elife*. 2015;4:e08881. [PubMed: 26650351]
58. Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, et al. The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTL/ALS. *Science*. 2013;339(6125):1335–8. [PubMed: 23393093]
59. Mizielinska S, Gronke S, Niccoli T, Ridler CE, Clayton EL, Devoy A, et al. C9orf72 repeat expansions cause neurodegeneration in *Drosophila* through arginine-rich proteins. *Science*. 2014;345(6201):1192–4. [PubMed: 25103406]
60. Schmidt EK, Clavarino G, Ceppi M, Pierre P. SUnSET, a nonradioactive method to monitor protein synthesis. *Nat Methods*. 2009;6(4):275–7. [PubMed: 19305406]
61. Kanekura K, Yagi T, Cammack AJ, Mahadevan J, Kuroda M, Harms MB, et al. Poly-dipeptides encoded by the C9ORF72 repeats block global protein translation. *Hum Mol Genet*. 2016;25(9):1803–13. [PubMed: 26931465]
62. Kwon MS, Noh MY, Oh KW, Cho KA, Kang BY, Kim KS, et al. The immunomodulatory effects of human mesenchymal stem cells on peripheral blood mononuclear cells in ALS patients. *J Neurochem*. 2014;131(2):206–18. [PubMed: 24995608]
63. Lee JE, Cooper TA. Pathogenic mechanisms of myotonic dystrophy. *Biochem Soc Trans*. 2009;37(Pt 6):1281–6. [PubMed: 19909263]

64. Meola G, Cardani R. Myotonic dystrophy type 2 and modifier genes: an update on clinical and pathomolecular aspects. *Neurol Sci.* 2017;38(4):535–46. [PubMed: 28078562]
65. Bates G. Huntingtin aggregation and toxicity in Huntington's disease. *Lancet.* 2003;361(9369):1642–4. [PubMed: 12747895]
66. Savas JN, Ma B, Deinhardt K, Culver BP, Restituto S, Wu L, et al. A role for Huntington disease protein in dendritic RNA granules. *J Biol Chem.* 2010;285(17):13142–53. [PubMed: 20185826]
67. de Mezer M, Wojciechowska M, Napierala M, Sobczak K, Krzyzosiak WJ. Mutant CAG repeats of Huntingtin transcript fold into hairpins, form nuclear foci and are targets for RNA interference. *Nucleic Acids Res.* 2011;39(9):3852–63. [PubMed: 21247881]
68. Grima JC, Daigle JG, Arbez N, Cunningham KC, Zhang K, Ochaba J, et al. Mutant Huntingtin disrupts the nuclear pore complex. *Neuron.* 2017;94(1):93–107.e6. [PubMed: 28384479]
69. Gasset-Rosa F, Chillon-Marinac C, Goginashvili A, Atwal RS, Artates JW, Tabet R, et al. Polyglutamine-expanded Huntingtin exacerbates age-related disruption of nuclear integrity and nucleocytoplasmic transport. *Neuron.* 2017;94(1):48–57.e4. [PubMed: 28384474]
70. Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell.* 1991;67(6):1047–58. [PubMed: 1760838]
71. Kremer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, et al. Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. *Science.* 1991;252(5013):1711–4. [PubMed: 1675488]
72. Yu S, Pritchard M, Kremer E, Lynch M, Nancarrow J, Baker E, et al. Fragile X genotype characterized by an unstable region of DNA. *Science.* 1991;252(5010):1179–81. [PubMed: 2031189]
73. Tassone F, Hagerman RJ, Chamberlain WD, Hagerman PJ. Transcription of the FMR1 gene in individuals with fragile X syndrome. *Am J Med Genet.* 2000;97(3):195–203. [PubMed: 11449488]
74. Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology.* 2001;57(1):127–30. [PubMed: 11445641]
75. Sofola OA, Jin P, Qin Y, Duan R, Liu H, de Haro M, et al. RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CGG premutation repeat-induced neurodegeneration in a Drosophila model of FXTAS. *Neuron.* 2007;55(4):565–71. [PubMed: 17698010]
76. Jin P, Duan R, Qurashi A, Qin Y, Tian D, Rosser TC, et al. Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a Drosophila model of fragile X tremor/ ataxia syndrome. *Neuron.* 2007;55(4):556–64. [PubMed: 17698009]
77. Sellier C, Freyermuth F, Tabet R, Tran T, He F, Ruffenach F, et al. Sequestration of DROSHA and DGCR8 by expanded CGG RNA repeats alters microRNA processing in fragile X-associated tremor/ataxia syndrome. *Cell Rep.* 2013;3(3):869–80. [PubMed: 23478018]
78. Todd PK, Oh SY, Krans A, He F, Sellier C, Frazer M, et al. CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome. *Neuron.* 2013;78(3):440–55. [PubMed: 23602499]
79. Oh SY, He F, Krans A, Frazer M, Taylor JP, Paulson HL, et al. RAN translation at CGG repeats induces ubiquitin proteasome system impairment in models of fragile X-associated tremor ataxia syndrome. *Hum Mol Genet.* 2015;24(15):4317–26. [PubMed: 25954027]
80. Sellier C, Buijsen RA, He F, Natla S, Jung L, Tropel P, et al. Translation of expanded CGG repeats into FMRpolyG is pathogenic and may contribute to fragile X tremor ataxia syndrome. *Neuron.* 2017;93(2):331–47. [PubMed: 28065649]
81. Maziuk B, Ballance HI, Wolozin B. Dysregulation of RNA binding protein aggregation in neurodegenerative disorders. *Front Mol Neurosci.* 2017;10:89. [PubMed: 28420962]
82. Iqbal K, Liu F, Gong CX, Grundke-Iqbal I. Tau in Alzheimer disease and related tauopathies. *Curr Alzheimer Res.* 2010;7(8):656–64. [PubMed: 20678074]
83. Vanderweyde T, Apicco DJ, Youmans-Kidder K, Ash PEA, Cook C, Lummertz da Rocha E, et al. Interaction of tau with the RNA-binding protein TIA1 regulates tau pathophysiology and toxicity. *Cell Rep.* 2016;15(7):1455–66. [PubMed: 27160897]

84. Díaz-Muñoz MD, Kiselev VY, Le Novère N, Curk T, Ule J, Turner M. Tial dependent regulation of mRNA subcellular location and translation controls p53 expression in B cells. *Nat Commun.* 2017;8(1):530. [PubMed: 28904350]
85. Gu J, Wu F, Xu W, Shi J, Hu W, Jin N, et al. TDP-43 suppresses tau expression via promoting its mRNA instability. *Nucleic Acids Res.* 2017;45(10):6177–93. [PubMed: 28335005]
86. Ding Q, Markesbery WR, Chen Q, Li F, Keller JN. Ribosome dysfunction is an early event in Alzheimer's disease. *J Neurosci.* 2005;25(40):9171–5. [PubMed: 16207876]
87. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. *Cell.* 2009;136(4):642–55. [PubMed: 19239886]
88. Kawahara Y, Mieda-Sato A. TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. *Proc Natl Acad Sci U S A.* 2012;109(9):3347–52. [PubMed: 22323604]
89. Porta S, Kwong LK, Trojanowski JQ, Lee VM. Drosha inclusions are new components of dipeptide-repeat protein aggregates in FTLTDP and ALS C9orf72 expansion cases. *J Neuropathol Exp Neurol.* 2015;74(4):380–7. [PubMed: 25756586]
90. Chou CC, Zhang Y, Umoh ME, Vaughan SW, Lorenzini I, Liu F, et al. TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in 1 ALS/FTD. *Nat Neurosci.* 2018;21(2):228–39. [PubMed: 29311743]
91. Eitan C, Hornstein E. Vulnerability of microRNA biogenesis in FTD-ALS. *Brain Res.* 2016;1647:105–11. [PubMed: 26778173]
92. Gascon E, Lynch K, Ruan H, Almeida S, Verheyden JM, Seeley WW, et al. Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemporal dementia. *Nat Med.* 2014;20(12):1444–51. [PubMed: 25401692]
93. Zhang Z, Almeida S, Lu Y, Nishimura AL, Peng L, Sun D, et al. Downregulation of microRNA-9 in iPSC-derived neurons of FTD/ALS patients with TDP-43 mutations. *PLoS One.* 2013;8(10):e76055. [PubMed: 24143176]
94. Rinchetti P, Rizzuti M, Faravelli I, Corti S. MicroRNA metabolism and dysregulation in amyotrophic lateral sclerosis. *Mol Neurobiol.* 2018;55(3):2617–30. [PubMed: 28421535]
95. Costa CJ, Willis DE. To the end of the line: axonal mRNA transport and local translation in health and neurodegenerative disease. *Dev Neurobiol.* 2018;78(3):209–20. [PubMed: 29115051]
96. Spaulding EL, Burgess RW. Accumulating evidence for axonal translation in neuronal homeostasis. *Front Neurosci.* 2017;11:312. [PubMed: 28620277]
97. Cestra G, Rossi S, Di Salvio M, Cozzolino M. Control of mRNA translation in ALS proteinopathy. *Front Mol Neurosci.* 2017;10:85. [PubMed: 28386218]
98. Shi Z, Fujii K, Kovary KM, Genuth NR, Rost HL, Teruel MN, et al. Heterogeneous ribosomes preferentially translate distinct subpools of mRNAs genome-wide. *Mol Cell.* 2017;67(1):71–83.e7. [PubMed: 28625553]
99. Ernst V, Levin DH, London IM. In situ phosphorylation of the alpha subunit of eukaryotic initiation factor 2 in reticulocyte lysates inhibited by heme deficiency, double-stranded RNA, oxidized glutathione, or the heme-regulated protein kinase. *Proc Natl Acad Sci U S A.* 1979;76(5):2118–22. [PubMed: 287050]
100. Moreno JA, Halliday M, Molloy C, Radford H, Verity N, Axten JM, et al. Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Sci Transl Med.* 2013;5(206):206ra138.
101. Briggs DI, Defensor E, Memar Ardestani P, Yi B, Halpain M, Seabrook G, et al. Role of endoplasmic reticulum stress in learning and memory impairment and Alzheimer's disease-like neuropathology in the PS19 and APPSwe mouse models of tauopathy and amyloidosis. *eNeuro.* 2017;4(4). 10.1523/ENEURO.0025-17.2017.
102. Johnson EC, Kang J. A small molecule targeting protein translation does not rescue spatial learning and memory deficits in the hAPP-J20 mouse model of Alzheimer's disease. *PeerJ.* 2016;4:e2565. [PubMed: 27781164]

Fig.11.1.

Translation in normal conditions and disease. **(a)** (i) The process of polypeptide synthesis begins with the attachment of the AUG start codon within the mRNA template to the small ribosomal subunit. A transfer RNA (tRNA) specific to the AUG codon positions a methionine (Met) residue on the P (Peptidyl) site of the small ribosomal subunit, forming a translation initiation complex with the aid of translation initiation factors. (ii) Elongation of the peptide chain occurs through the A (Acceptor) site where a tRNA specific to the next codon within the mRNA template recruits the proper amino acid, which is bound to the initial Met at the P site with a peptide bond. (iii) Using this stepwise strategy, the peptide growth continues in an elongation loop until a stop codon (UAA, UAG, or UGA) is reached, causing specialized proteins called release factors to free the mRNA template as well as the newly formed polypeptide. **(b)** Non-AUG Initiated Translation. (i) Internal Ribosome Entry Site (IRES) translation generates normal polypeptide chains. (ii) Repeat Associated Non-AUG (RAN) translation forms toxic dipeptides. **(c)** Errors in translation. (i) Sequestration of mRNA in protein-mRNA granules prevents integration into a ribosome complex and therefore translation. (ii) Trafficking proteins are necessary for proper mRNA trafficking; their absence or dysfunction leads to a lack of mRNA in specific cellular locations. (iii) Deficient synthesis of tRNA prevents polypeptide addition, even with proper translation initiation. (iv) Deficits in ribogenesis reduce the number of actively translating ribosomal complexes

Fig.11.2.

Tagged ribosome affinity purification. **(a, b)** Model system-specific expression systems allow expression of tagged ribosomal subunit (RPL10-GFP) to be specifically expressed in cell types of interest (motor neurons or glia, green). **(c)** Using anti-GFP antibodies, the tagged ribosomal subunits are immunoprecipitated out of the whole body lysate. **(d)** Immunoprecipitated mRNA is isolated and subjected to RNA sequencing and bioinformatics to identify cell-specific translomes normalized to input mRNA levels

Fig.11.4. Causes of Translational Inhibition. Cells regulate translation through a robust, complex integration of multiple pathways. The dysregulation of such pathways can alter proteostasis within a cell and lead to neuronal dysfunction