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Review

Converging Mechanisms in ALS and FTD: Disrupted RNA and Protein Homeostasis

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Breakthrough discoveries identifying common genetic causes for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) have transformed our view of these disorders. They share unexpectedly similar signatures, including dysregulation in common molecular players including TDP-43, FUS/TLS, ubiquilin-2, VCP, and expanded hexanucleotide repeats within the *C9ORF72* gene. Dysfunction in RNA processing and protein homeostasis is an emerging theme. We present the case here that these two processes are intimately linked, with disease-initiated perturbation of either leading to further deviation of both protein and RNA homeostasis through a feedforward loop including cell-to-cell prion-like spread that may represent the mechanism for relentless disease progression.

Introduction

Amyotrophic lateral sclerosis (ALS, familiarly known in the United States as Lou Gehrig's disease) was first reported 140 years ago by the great French physician Jean-Martin Charcot. The name describes the key features of the disease: muscle wasting (amyotrophic) due to the degeneration of lower motor neurons and their axons and loss of upper motor neurons and their corticospinal axonal tracts (lateral sclerosis). In contrast to ALS, frontotemporal dementia (FTD) (also known as frontotemporal lobar degeneration [FTLD]) is a progressive neuronal atrophy with loss in the frontal and temporal cortices and is characterized by personality and behavioral changes, as well as gradual impairment of language skills. It is the second most common dementia after Alzheimer's disease (Van Langenhove et al., 2012).

Here, we review the key findings that have revealed a tangled web in which multiple pathways are involved in disease initiation and progression in ALS and FTD. RNA and protein homeostasis pathways are intimately linked and their dysfunction is fundamentally involved in disease pathogenesis. Perturbation of either pathway can amplify an initial abnormality through a feedforward loop, which may underlie relentless disease progression.

Convergence of Pathogenic Mechanisms of ALS and FTD

Largely indistinguishable, familial (10%) and sporadic (90%) ALS are characterized by premature degeneration of upper and lower motor neurons. Mutations in four genes (*C9ORF72*, *SOD1*, *TARDBP*, and *FUS*/*TLS*) account for over 50% of the familial cases (Table S1 available online). For FTD, a stronger genetic contribution is reflected by the higher percentage (up to 50%) of patients with a familial history. This includes the first two identified causal genes encoding the microtubule-associated protein tau (*MAPT*) (Hutton et al., 1998) and progranulin (*PGRN*) (Baker et al., 2006; Cruts et al., 2006), which together account for 10%–20% of FTD (Van Langenhove et al., 2012). More rarely, mutations in TDP-43 and FUS/TLS are causal for FTD (reviewed in Lagier-Tourenne et al., 2010; Mackenzie et al., 2010a). Recently, hexanucleotide expansion in the *C9ORF72* gene was found to be a common genetic cause for ALS and FTD (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011) (Table S1).

It is estimated that 15% of FTD patients meet ALS criteria (Ringholz et al., 2005), and ALS can be accompanied by cognitive and behavioral impairment, with perhaps as much as 15% of affected individuals also developing symptoms consistent with a typical definition of FTD (Ringholz et al., 2005; Wheaton et al., 2007). ALS and FTD are linked clinically, pathologically, and mechanistically, and the diseases are now properly recognized as representatives of a continuum of a broad neurodegenerative disorder, with each presenting in a spectrum of overlapping clinical symptoms (Figure 1).

A breakthrough linking disease mechanisms for ALS and FTD came with the identification of TDP-43 as the major ubiquitinated protein found in both sporadic ALS patients and the most frequent pathological form of FTD (Arai et al., 2006; Neumann et al., 2006). This finding was followed by the discovery of mutations in the gene encoding the RNA-binding protein TDP-43 in ~5% of familial ALS cases (Kabashi et al., 2008; Sreedharan et al., 2008; Van Deerlin et al., 2008) and rare patients with FTD (Borroni et al., 2009; Kovacs et al., 2009).

Recognition that errors in RNA-binding proteins are causative of ALS and FTD was quickly expanded, with mutations in the fused in sarcoma/translocated in liposarcoma (*FUS*/*TLS*) gene shown to account for an additional ~5% of familial ALS and also rare cases of FTD (Kwiatkowski et al., 2009; Vance et al., 2009). Subsequent confirmation that FUS/TLS was present in the pathological inclusions in most of the FTD patients without TDP-43-containing inclusions has led to a proposed

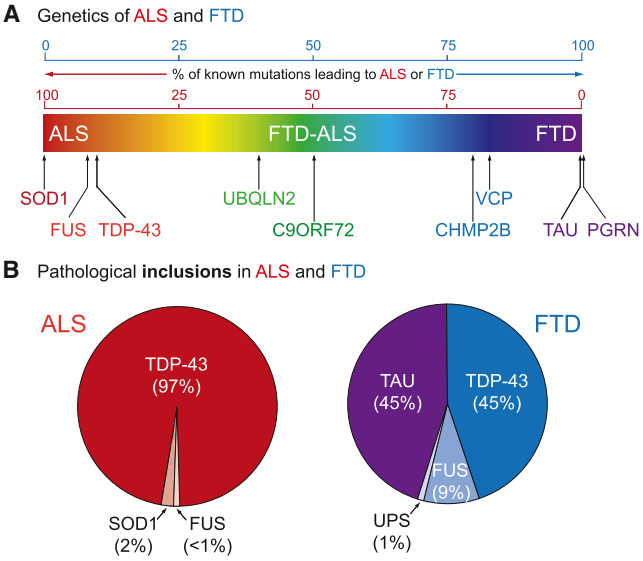

Figure 1. Clinical, Genetic, and Pathological Overlap of ALS and FTD

(A) ALS and FTD represent a continuum of a broad neurodegenerative disorder with each presenting as extremes of a spectrum of overlapping clinical symptoms (ALS in red and FTD in purple). Major known genetic causes for ALS and FTD are plotted according to the ratio of known mutations that give rise to ALS or FTD.

(B) Pathological protein inclusions in ALS and FTD, according to the major protein misaccumulated. Inclusions of TDP-43 and FUS/TLS in ALS and FTD reflect the pathological overlap of ALS and FTD.

reclassification of FTD based on the main protein component accumulated (Mackenzie et al., 2010b; Sieben et al., 2012). These include FTLD-tau (45%), FTLD-TDP (45%), FTLD-FUS (9%), and a remaining 1% named FTLD-UPS (for ubiquitin-proteasome system) (Figure 1). Altogether, these findings highlight two main discoveries: (1) TDP-43 and FUS/TLS, both RNA-binding proteins linked to multiple steps of RNA metabolism, are the major protein components of pathological inclusions observed in over 90% of ALS and over 50% of FTD patients, and (2) errors in RNA processing may be central to ALS and FTD pathogenesis.

A further direct molecular link between ALS and FTD was identification of a large intronic hexanucleotide expansion (~400–1,600 GGGGCC repeats) in the previously uncharacterized gene *C9ORF72* (named for its location on chromosome 9, open reading frame 72) in families with either ALS, FTD, or both (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011). The expanded repeat in *C9ORF72* is reminiscent of previously studied repeat expansion diseases (La Spada and Taylor, 2010), especially myotonic dystrophy and fragile X mental retardation syndrome, whose precedents support at least two possible pathogenic mechanisms: RNA-mediated toxicity or haploinsufficiency.

ALS-, ALS/dementia-, and/or FTD-causing mutations were also identified in genes involved in protein clearance pathways or maintaining proper protein homeostasis, including ubiquilin-2 (*UBQLN2*) (Deng et al., 2011), vasolin-containing protein (*VCP*) (Johnson et al., 2010; Watts et al., 2007), vesicle-associated membrane protein-associated protein B (*VAPB*) (Nishimura et al., 2004), p62/sequestosome (*SQSTM1*) (Fecto et al., 2011; Rubino et al., 2012; Teyssou et al., 2013), optineurin (*OPTN*) (Maruyama et al., 2010), and charged multivesicular body protein 2B or chromatin modifying protein 2B (*CHMP2B*) (Parkinson et al., 2006; Skibinski et al., 2005). Coupled with protein aggregation as a major pathological hallmark of both ALS and FTD, the genetic discoveries indicate that disruption in protein homeostasis (or proteostasis) is a key characteristic of both diseases.

ALS- and FTD-Linked Genes Disrupt RNA Homeostasis

Identification of disease-linked mutations in TDP-43 and FUS/TLS marked the beginning of a paradigm shift, highlighting dysfunctions in RNA metabolism as a central pathogenic pathway in ALS and FTD. TDP-43 and FUS/TLS share similar structural and functional properties with probable involvement in multiple RNA processing steps (Lagier-Tourenne et al., 2010). ALS-linked mutations have been identified in genes encoding *TAF15* (TATA-binding protein-associated factor 15) (Couthouis et al., 2011; Ticozzi et al., 2011) and *EWSR1* (Ewing's sarcoma breakpoint region 1) (Couthouis et al., 2012), two proteins that are functionally and structurally similar to *FUS/TLS*, albeit the mutations have not been proven to be causative of disease. Altogether, with additional ALS-linked mutations in the RNA-binding proteins angiogenin (Greenway et al., 2006), senataxin (Chen et al., 2004), and ataxin-2 (Elden et al., 2010), disruption in RNA homeostasis seems highly likely to play a central role in ALS pathogenesis.

TDP-43 and FUS/TLS Reshape ALS and FTD

TDP-43 Mutation and Pathology in ALS and FTD

TDP-43 is a 414 amino acid protein containing two RNA recognition motifs (RRMs) followed by a glycine-rich, low-sequence complexity prion-like domain (Kato et al., 2012; King et al., 2012). TDP-43 can shuttle between the cytosol and the nucleus (Ayala et al., 2008; Winton et al., 2008), although the majority of TDP-43 appears to be nuclear in most cells at steady state. Pathological inclusions of TDP-43 can be found in the nucleus and cytosol of neurons and glia, with abnormal phosphorylation and ubiquitination of TDP-43 and the presence of truncated C-terminal fragments (Arai et al., 2006; Neumann et al., 2006). More than 40 mutations in sporadic and familial ALS, as well as in rare cases of FTD (reviewed in Lagier-Tourenne et al., 2010; Lattante et al., 2013), are found clustered within the prion-like domain (so named because of its similarity to fungal prions) (Figure S1).

In the absence of mutation, TDP-43 pathology can be found in the majority of ALS patients, with the exception of patients with SOD1 mutations (Mackenzie et al., 2007; Tan et al., 2007), and is apparently indistinguishable between patients with or without TDP-43 mutations (Pamphlett et al., 2009). Cells with TDP-43 aggregates typically have concomitant loss of nuclear TDP-43, indicating loss of nuclear TDP-43 function, while the presence of cytoplasmic protein inclusions suggests gain of one or more toxic properties. Thus, the pathogenic mechanisms for TDP-43 are likely to be a combination of both loss-of-function and gain-of-toxic properties.

Normal Function of TDP-43

TDP-43 was first identified as a protein that bound to the transactivation response (TAR) element of HIV human immunodeficiency virus and was named TAR DNA-binding protein-43 kDa.

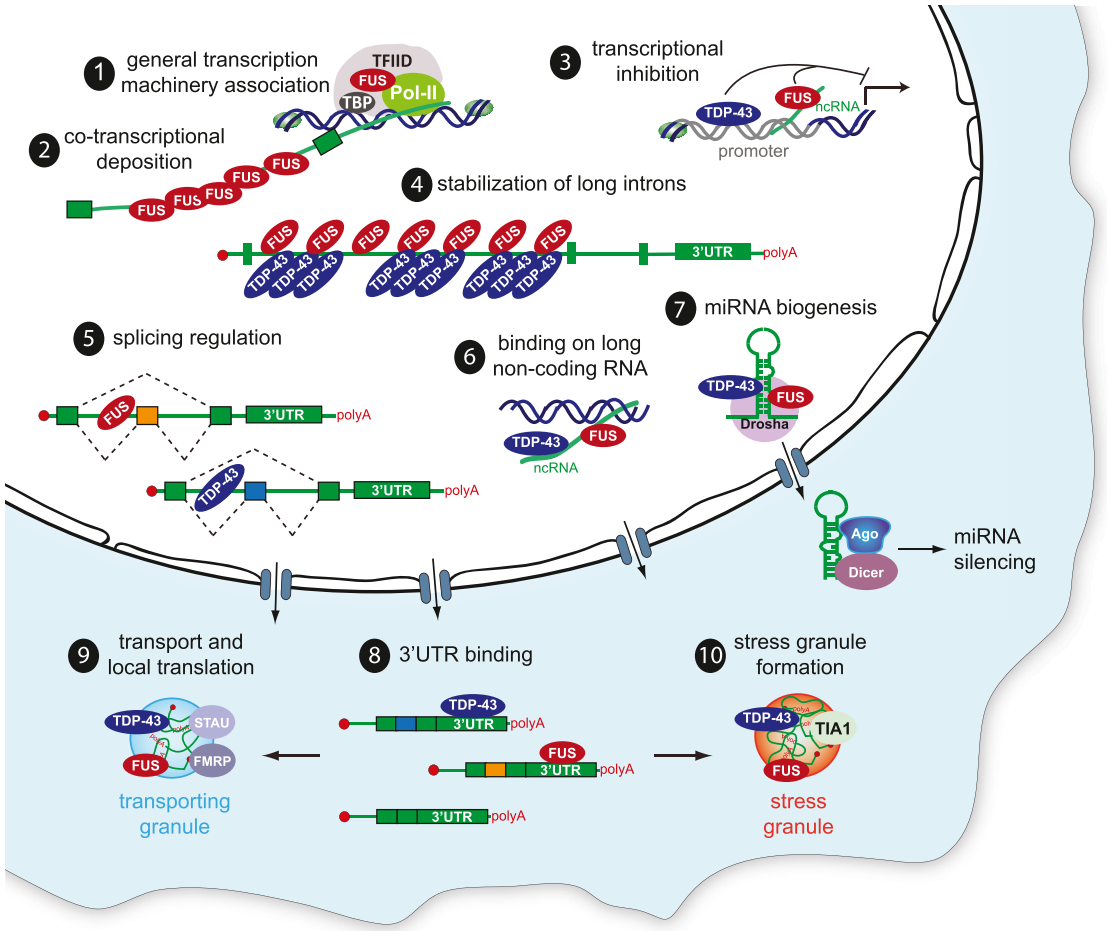

Figure 2. Physiological Roles of TDP-43 and FUS/TLS in RNA Processing

Proposed roles for FUS/TLS include (1) association with TBP within the TFIID complex as a participant in the general transcriptional machinery and (2) binding to long introns in a sawtooth-like pattern, consistent with cotranscriptional deposition. Both TDP-43 and FUS/TLS (3) associate with promoter regions. TDP-43 binds single-stranded TG-rich elements in promoter regions, thereby blocking transcription of the downstream gene. In response to DNA damage, FUS/TLS is recruited in the promoter region of cyclin D1 (CCND1) by sense and antisense noncoding RNAs (ncRNAs) and represses CCND1 transcription. BothTDP-43 and FUS/TLS (4) bind long intron-containing RNAs, thereby sustaining their levels. (5) TDP-43 and FUS/TLS control the splicing of >950 or >370 RNAs, respectively, either via direct binding or indirectly. TDP-43 and FUS/TLS (6) bind long noncoding RNAs, (7) complex with Drosha (consistent with an involvement in miRNA processing), and (8) bind 3′UTRs of a large number of mRNAs. Both TDP-43 and FUS/TLS shuttle between the nucleus and the cytosol and are incorporated into (9) transporting RNA granules and (10) stress granules, in which they form complexes with mRNAs and other RNA-binding proteins.

TDP-43 can act as a transcriptional repressor and is associated with proteins involved in transcription (Ling et al., 2010; Sephton et al., 2011), including methyl CpG-binding protein 2 (MeCP2) (Sephton et al., 2011), whose mutations are causative for Rett syndrome. Genome-wide approaches are now needed to identify the complete set of genes for which TDP-43 plays a transcriptional role through its direct DNA binding. TDP-43 is involved in many aspects of RNA-related metabolism, including splicing, microRNA (miRNA) biogenesis, RNA transport and translation, and stress granule formation by interacting with numerous hnRNPs, splicing factors, and microprocessor proteins (reviewed in Buratti and Baralle, 2012; Lagier-Tourenne et al., 2010; Polymenidou et al., 2012) (Figure 2A).

TDP-43's RNA Targets

An unbiased genome-wide approach was used to identify the in vivo RNA targets for TDP-43 in mouse (Polymenidou et al., 2011) and human (Tollervey et al., 2011) brain. More conventional methodology has also been used in an effort to identify RNA targets of TDP-43 in rat cortical neurons (Sephton et al., 2011), a mouse NSC-34 cell line (Colombrita et al., 2012), and a human neuroblastoma cell line (Xiao et al., 2011). It is clear that TDP-43 binds to more than 6,000 RNA targets in the brain, roughly 30% of the total transcriptome (Figure 3). The localization of TDP-43's binding sites across different pre-mRNAs reveals its various roles in RNA maturation. Indeed, intronic binding of TDP-43 on long-intron (>100 kb)-containing RNA targets was shown to be required for sustaining their normal levels (Polymenidou et al., 2011). Splice site selection may be influenced by TDP-43 binding near exon-intron junctions as well as in the intronic regions far away (>2 kb) from the nearest exon (Polymenidou et al., 2011; Tollervey et al., 2011). In addition, TDP-43 binding on the 3′UTR of mRNAs may affect their stability or transport,

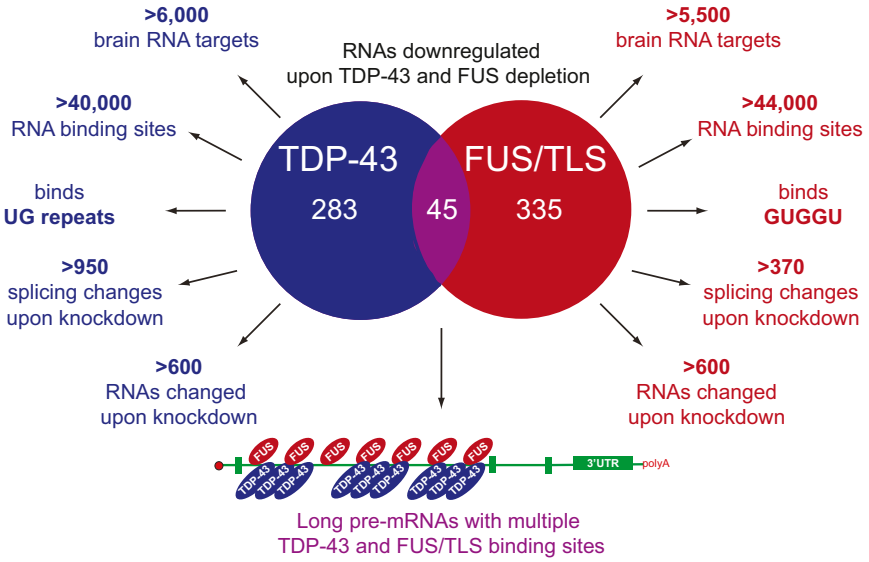

Figure 3. Comparison of TDP-43 and FUS/TLS RNA-Binding Properties

Data are taken from Polymenidou et al. (2011), Lagier-Tourenne et al. (2012), Tollervey et al. (2011), Sephton et al. (2011), Colombrita et al. (2012), Rogelj et al. (2012), Hoell et al. (2011), Ishigaki et al. (2012), and Nakaya et al. (2013).

while TDP-43 binding on long noncoding RNAs (ncRNAs) may influence their regulatory roles.

TDP-43 levels matter greatly for normal RNA maturation. Antisense oligonucleotide-mediated reduction of TDP-43 within an otherwise normal mouse nervous system affects the levels of more than 600 mRNAs and the splicing pattern of another ~950 (Polymenidou et al., 2011). TDP-43 also binds to the 3′UTRs of more than 1,000 transcripts (Polymenidou et al., 2011; Tollervey et al., 2011), including its own mRNA, presumably affecting nuclear or cytoplasmic RNA stability. It also has binding sites on many ncRNAs whose functions are not yet clearly defined but include chromatin remodeling, transcription regulation, and posttranscriptional processing. Among these, TDP-43 binds to long (>200 base) ncRNAs, including nuclear-enriched autosomal transcript 1 (NEAT1) and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (Tollervey et al., 2011). Expression of both NEAT1 and MALAT1 is elevated in FTD-TDP (also known as FTLD-TDP) patients, which correlates with increased TDP-43 association with both ncRNAs (Tollervey et al., 2011). These data suggest that TDP-43 may affect RNA metabolism, including >300 mRNAs without TDP-43 binding sites but whose abundance increases through an indirect mechanism when TDP-43 is reduced (Polymenidou et al., 2011).

The binding of TDP-43 to small (<200 base) ncRNAs and miRNAs remains largely unexplored. Nonetheless, the association of TDP-43 with Drosha microprocessor (Ling et al., 2010) and Dicer complexes (Freibaum et al., 2010; Kawahara and Mieda-Sato, 2012) is suggestive of a TDP-43 involvement in miRNA biogenesis. Indeed, let-7b miRNA is downregulated, whereas miR-663 is upregulated after reduction in TDP-43 (Buratti et al., 2010).

FUS/TLS Mutation and Pathology in ALS and FTD

ALS/FTD-linked mutations in FUS/TLS are clustered into two groups: mutations in the low-complexity/prion-like domain and mutations in the C-terminal nuclear localization signal (NLS) (Figure S1). Mutations in the latter group typically lead to increased cytoplasmic localization of FUS/TLS (Kwiatkowski et al., 2009; Vance et al., 2009) and several are associated with juvenile onset ALS (Bäumer et al., 2010; Belzil et al., 2012; Huang et al., 2010; Yamashita et al., 2012).

Distinct patterns of FUS pathology have been correlated with disease severity and mutation (Mackenzie et al., 2011). Early-onset ALS cases are characterized by basophilic inclusions and round neuronal cytoplasmic FUS inclusions, whereas late-onset ALS cases are characterized by tangle-like FUS-containing inclusions in both neurons and glial cells. FUS inclusions in the absence of FUS mutations have also been reported in FTD, Huntington's disease, and spinocerebellar ataxia 1 and 2 (reviewed in Lagier-Tourenne et al., 2010).

Normal Function of FUS/TLS

Similar to TDP-43, FUS/TLS can bind to single- and double-stranded DNA, as well as RNA, and almost certainly participates in a wide range of cellular processes (Lagier-Tourenne et al., 2010; Tan and Manley, 2009).

Transcription. All three members of the FET (FUS/TLS, EWSR1, and TAF-15) family have been shown to associate with RNA polymerase II (RNAP II) and its general transcription factor TFIID. FUS/TLS and TAF15 fractionate with different populations of TFIID complexes, suggesting that they may affect different promoters (Bertolotti et al., 1996). It is likely that FUS/TLS can affect the transcription of specific genes through its association with several nuclear hormone receptors (Powers et al., 1998) and gene-specific transcription factors. Indeed, a recent study identified potential FUS/TLS-response elements of many target genes, indicative of transcriptional activation or repression directly by FUS/TLS (Tan et al., 2012). FUS/TLS can also associate with TBP and TFIIIB to repress transcription by RNAP III, which transcribes small structural and catalytic RNAs (Tan and Manley, 2010).

Splicing. FUS/TLS has been identified as part of the spliceosome machinery in three independent proteomic studies (Hartmuth et al., 2002; Rappsilber et al., 2002; Zhou et al., 2002). The association of FUS/TLS with the spliceosome and various splicing factors initially implicated FUS/TLS in a cotranscriptional role and/or splicing regulation of pre-mRNAs, a prediction validated by demonstration that about 1,000 RNAs change in splicing pattern or abundance in a FUS/TLS-dependent manner in the mouse brain (Lagier-Tourenne et al., 2012) (Figure 3).

FUS/TLS's RNA Targets

Genome-wide approaches (summarized in Figure 3) have identified more than 8,000 in vivo RNA targets for FUS/TLS in mouse

(Lagier-Tourenne et al., 2012; Rogelj et al., 2012), 5,500 in human (Lagier-Tourenne et al., 2012), and more than 6,800 in various cell lines (Colombrita et al., 2012; Hoell et al., 2011; Ishigaki et al., 2012; Nakaya et al., 2013). A GUGGU sequence is the most prominent binding motif (Lagier-Tourenne et al., 2012). In addition, AU-rich stem loops bound by FUS/TLS have also been proposed (Hoell et al., 2011). A sawtooth-like binding pattern to long introns (Lagier-Tourenne et al., 2012; Rogelj et al., 2012) is consistent with cotranscriptional deposition of FUS/TLS and suggests that FUS/TLS remains bound to pre-mRNAs until splicing is completed. In addition, FUS/TLS shows enrichment in binding to 3′UTRs and exons.

Interestingly, RNAs bound by TDP-43 and FUS/TLS are largely distinct (Lagier-Tourenne et al., 2012; Rogelj et al., 2012). Indeed, depletion of FUS/TLS from an otherwise normal adult mouse nervous system alters levels or splicing of >970 mRNAs, most of which are distinct from RNAs dependent on TDP-43. Remarkably, only 45 RNAs are reduced upon depletion of either TDP-43 or FUS/TLS from mouse brain, including mRNAs transcribed from genes with exceptionally long introns and that encode proteins essential for neuronal integrity (Lagier-Tourenne et al., 2012). A subset of these is significantly lowered after TDP-43 or FUS/TLS depletion in stem cell-derived human neurons and in TDP-43 aggregate-containing motor neurons in sporadic ALS, evidence pointing to a common loss-of-function pathway as one component underlying motor neuron death from misregulation of TDP-43 or FUS/TLS (Lagier-Tourenne et al., 2012).

Cytoplasmic Functions of TDP-43 and FUS/TLS

TDP-43 and FUS/TLS in Cytoplasmic RNA Granules

TDP-43 and FUS/TLS shuttle from the nucleus to the cytosol (Ayala et al., 2008; Zinszner et al., 1997), where they have been associated with cytoplasmic RNA granules that contain non-translating mRNAs. These granules include processing bodies (P bodies), which contain RNA decay machinery (Buchan and Parker, 2009), stress granules, which contain translation machinery (Anderson and Kedersha, 2009), and transporting RNP granules, which contain RNAs to be locally translated (Kiebler and Bassell, 2006).

TDP-43 and FUS/TLS at the Synapse

Deletion of FUS/TLS has produced abnormal dendritic and spine morphology in cultured hippocampal neurons (Fujii et al., 2005). Evidence suggests that FUS/TLS may play an important role in regulating synaptic function, possibly through local transport and translation. In dendrites of cultured hippocampal neurons, TDP-43 has been shown to colocalize with fragile X mental retardation protein (FMRP) and staufen, two proteins that mark transporting RNP granules and P bodies (Wang et al., 2008). Given the evidence that TDP-43 and FUS/TLS bind to many RNA targets important for synaptic function and that TDP-43 and FUS/TLS localize to dendrites in response to neuronal activation (Fujii et al., 2005; Wang et al., 2008), dysfunction of TDP-43 or FUS/TLS is highly likely to alter synaptic function.

Assembly of RNA Granules through Prion-like Domains

Both TDP-43 and FUS/TLS contain low-sequence complexity (LC), fungal prion-like domains (King et al., 2012), for which a normal function in RNA granule assembly has recently been proposed (Han et al., 2012b; Kato et al., 2012). Assembly of the LC domain of FUS/TLS produces amyloid-like fibers that, in contrast to pathological amyloid inclusions, are reversible (Kato et al., 2012). Induced assembly of LC domains—along with their linked RNA-binding domains—provides a basis for RNA granule assembly and possibly for cell-to-cell spreading.

Disease Mechanisms for Mutant TDP-43 and FUS/TLS

Evidence for Gain of Toxicity from Mutant TDP-43

Multiple transgenic approaches have been employed to identify properties of mutant TDP-43. We focus here only on mammalian models; readers are directed to excellent reviews elsewhere on yeast, *Drosophila*, *C. elegans*, and other animal models (Da Cruz and Cleveland, 2011; Joyce et al., 2011; McGoldrick et al., 2013). It should be acknowledged that the multiple efforts that have produced TDP-43 transgenic mice and rats have—for the most part—been disappointing. One effort (with a prion-promoted TDP-43^{Q331K}) did produce age-dependent, mutant-dependent motor neuron disease in which about half of the lower motor neurons died, but disease then plateaued despite continued mutant TDP-43 accumulation at a constant level (Arnold et al., 2013). Mutant TDP-43-dependent degeneration of lower (but not upper) motor neurons occurred without loss of nuclear TDP-43 or accumulation of TDP-43 aggregates but was accompanied by both loss and gain of splicing function of selected RNA targets at an early disease stage. Thus, disease mechanism is apparently both gain of aberrant property and loss of function. Inexplicably, a similar prion-promoted transgenic line (TDP-43^{A315T}) develops disease with very different characteristics: upper motor neuron loss (Wegorzewska et al., 2009) with very modest lower motor neuron disease, prior to death from bowel obstruction (Esmaeili et al., 2013; Guo et al., 2012).

Additional TDP-43 transgenic efforts have established that increased TDP-43 levels (by less than a factor of 2) of either wild-type or mutant TDP-43 are highly deleterious (Igaz et al., 2011; Wils et al., 2010). This has revealed a crucial role for an autoregulatory pathway that maintains TDP-43 RNA levels. Evidence for autoregulation of TDP-43 has been repeatedly seen: inactivation of one copy of TDP-43 in mice does not affect either the mRNA or protein level of TDP-43 (Kraemer et al., 2010; Sephton et al., 2010). Autoregulation is mediated, at least in part, by TDP-43-dependent splicing of an intron in the 3′UTR of its own mRNA (Avendaño-Vázquez et al., 2012; Ayala et al., 2011b; Polymenidou et al., 2011). Splicing of this intron generates an unstable RNA degraded by nonsense-mediated decay (Polymenidou et al., 2011). An additional proposal is that this TDP-43-dependent 3′UTR splicing event activates a cryptic polyadenylation site whose use leads to nuclear retention of TDP-43 RNA (Avendaño-Vázquez et al., 2012).

Increasing TDP-43 levels in mice and rats (by expression of RNAs missing the autoregulatory sequences (Wegorzewska et al., 2009; Wils et al., 2010; Igaz et al., 2011; Arnold et al., 2013) or by disrupting autoregulation (Igaz et al., 2011) has produced neurodegeneration. The level of expression determines the severity of disease (e.g., Wils et al., 2010; Igaz et al., 2011; Arnold et al., 2013). Mice expressing autoregulated wild-type and ALS-linked mutant genomic TDP-43 transgenes develop very mild, late-onset cognitive and motor deficits but without paralysis (Swarup et al., 2011). Age-dependent, mutant-dependent

motor neuron disease develops with TDP-43^{Q331K} accumulating to a level similar to the normal level of endogenous TDP-43 (Arnold et al., 2013). Expression of genes missing the autoregulatory 3′UTR—thereby permitting accumulation of mutant TDP-43^{M337V} (to an undetermined level)—drives paralysis in rats within 35 days after inducing transgene expression broadly (Zhou et al., 2010) or within 15 days when the transgene is induced panneuronally (Huang et al., 2012).

TDP-43 Loss of Function in Disease

Loss of nuclear function of TDP-43 is clearly a component of the disease process, as nuclear clearing accompanied by cytoplasmic accumulation of TDP-43 has been universally reported in surviving neurons in patients with TDP-43 mutant-mediated ALS (Van Deerlin et al., 2008). Not unexpectedly, TDP-43 is an essential gene in mice, yielding embryonic lethality (Chiang et al., 2010; Kraemer et al., 2010; Sephton et al., 2010; Wu et al., 2010), while TDP-43 heterozygote mice are viable and fertile with autoregulation maintaining nearly normal TDP-43 levels (Kraemer et al., 2010).

Ubiquitous postnatal removal of TDP-43 through conditional TDP-43 gene inactivation produced rapid lethality without motor neuron disease (Chiang et al., 2010). Selective removal of TDP-43 from motor neurons produced age-dependent progressive motor neuron degeneration with ALS-like pathology, although in one study the mice lived a normal life span (Iguchi et al., 2013) and in the other study only the male mice developed pathology and phenotype (Wu et al., 2012). These observations are consistent with the notion that while neuronal loss of function of TDP-43 may contribute to disease development and progression, it is insufficient to produce fatal motor neuron disease.

TDP-43's RNA Targets and Disease Pathogenesis

Among the more than 6,000 RNAs normally bound by TDP-43—and the 1,500 who are changed in abundance or splicing pattern when nuclear TDP-43 is depleted (Figure 3)—are TDP-43 itself, FUS/TLS, glial excitatory amino acid transporter-2 (*EAAT2*), amyloid beta precursor protein (*APP*), presenilin, huntingtin, multiple ataxins, α-synuclein, progranulin, and tau (Polymenidou et al., 2011; Sephton et al., 2011). The most prominently affected class of RNAs are pre-mRNAs with exceptionally long introns (>100 kb), whose expression is enriched in brain and whose encoded proteins are involved in synaptic activity and functions, including parkin 2 (*PARK2*), neurexin 1 and 3 (*NRXN1* and *NRXN3*), and neuroligin 1 (*NLGN1*), whose mutations are associated with various neurological diseases.

Additionally, among the >600 RNAs whose splicing patterns are altered when TDP-43 levels are reduced are FUS/TLS itself and EAAT2, with expression of the latter also reduced in FTD-TDP brain (Tollervey et al., 2011). Many ALS-linked genes, including *ALSIN*, *CHMP2B*, *FIG4*, *VAPB*, and *VCP*, are bound by TDP-43, and their expression is modestly altered upon TDP-43 depletion (Polymenidou et al., 2011). TDP-43 also regulates the splicing of sortilin, a tentative receptor for progranulin (Hu et al., 2010), whose mutations are linked to FTD-TDP. Misregulation of sortilin splicing by reduction in TDP-43 affects progranulin metabolism (Prudencio et al., 2012), further suggesting that dysfunction of TDP-43 underlies FTD pathogenesis. Collectively, deregulation of TDP-43 RNA targets supports loss of nuclear TDP-43 function as a plausible contributor to pathogenesis after an initiating stress leading to cytoplasmic TDP-43 accumulation.

Mechanism(s) of ALS-Linked FUS/TLS Mutants

FUS Loss of Function in Disease

Like TDP-43, loss of nuclear function of FUS/TLS is also a likely component of the disease process, as nuclear clearing accompanied by cytoplasmic accumulation of FUS/TLS was initially reported in surviving neurons of patients with NLS mutant-mediated FUS/TLS (Kwiatkowski et al., 2009; Vance et al., 2009). Two independent FUS/TLS knockout mouse models have been generated (Kuroda et al., 2000; Hicks et al., 2000). Conflicting results from these models have made it unclear whether FUS/TLS is an essential gene.

FUS/TLS Mutant Gain of Toxicity

No currently published mouse model stably express ALS-linked mutations in FUS/TLS. However, one study in rats with inducible expression of human wild-type or R521C mutant of FUS/TLS reported that postnatal induction (to undetermined levels) in two independent lines of mutant-expressing rats produced paralysis and death by 70 days of age, whereas comparable wild-type human FUS/TLS-expressing rats survived normally (Huang et al., 2011). These findings support a gain of toxicity by mutant FUS/TLS, albeit rats overexpressing wild-type FUS/TLS also develop motor and spatial learning deficits accompanied by ubiquitin aggregation by 1 year of age. It should be noted that, similar to the case of TDP-43, increased wild-type FUS/TLS accumulation through homozygous mating in mice is also highly deleterious, driving early lethality (Mitchell et al., 2013). Additional mouse and rat models and further studies are needed to elucidate FUS/TLS-mediated toxicity.

Is TDP-43 and FUS/TLS-Mediated Toxicity a Non-Cell-Autonomous Process?

An increasing body of evidence has established that cell types beyond the target neurons whose dysfunction is responsible for the primary phenotypes also contribute to neurodegeneration, a phenomenon known as non-cell-autonomous toxicity (Garden and La Spada, 2012). Given that TDP-43 and FUS/TLS inclusion can also be found in glia (Mackenzie et al., 2010a), it is conceivable that glia contribute to disease pathogenesis. Indeed, induced pluripotent stem cell (iPSC)-derived astrocytes from patients carrying a familial mutation in TDP-43 (M337V) showed several abnormalities, including increased TDP-43 accumulation and altered subcellular localization (Serio et al., 2013). While these mutant astrocytes did not produce short-term toxicity to cocultured motor neurons, driving expression only in astrocytes of the same TDP-43 mutation (M337V) produced progressive loss of motor neurons and paralysis in rats (Tong et al., 2013). Thus, it is highly plausible that TDP-43 (and possibly FUS/TLS as well) mediated neurodegeneration is a non-cell-autonomous process.

TDP-43, FUS/TLS, and a Potential Link between Stress Granules and Protein Inclusion

TDP-43 and FUS/TLS are components of stress granules (Dewey et al., 2012; Li et al., 2013). The main functions of stress granules appear to be in temporally repressing general

translation and storage of mRNAs during stress. Importantly, stress granules are disassembled when the stressors are removed (Anderson and Kedersha, 2009).

At least seven independent studies have reported TDP-43 to be localized within stress granules produced in a wide range of cell lines with varying stresses, including oxidative, osmotic, and heat stresses (Ayala et al., 2011a; Colombrita et al., 2009; Dewey et al., 2011; Freibaum et al., 2010; Liu-Yesucevitz et al., 2010; McDonald et al., 2011; Meyerowitz et al., 2011). TDP-43 variants with ALS-linked mutations appear to form larger stress granules with faster kinetics (Dewey et al., 2011; Liu-Yesucevitz et al., 2010) and this requires the prion-like domain (Bentmann et al., 2012; Dewey et al., 2011; Liu-Yesucevitz et al., 2010).

Similarly, FUS/TLS is recruited into stress granules (Andersson et al., 2008) and FUS/TLS with ALS-linked mutations in its NLS show enhanced propensity to associate with stress granules (Bosco et al., 2010a; Dormann et al., 2010; Gal et al., 2011; Ito et al., 2011; Kino et al., 2011). One provocative report claimed that the prion-like domain of FUS/TLS is both necessary and sufficient to form stress granules in cultured cells and to form hydrogels in vitro (Kato et al., 2012). Another report claimed a completely opposite result, with the C-terminal residues together with an ALS-linked mutation (P525L), but not the prion-like domain, required for stress granule formation in cells (Bentmann et al., 2012). The discrepancy remains unresolved.

Nonetheless, the evidence collectively indicates that association of TDP-43 and FUS/TLS into stress granules is a normal physiological response to stress. A tempting speculation is that the association of TDP-43 and FUS/TLS with stress granules may be an initiating event, which following chronic stress eventually leads to irreversible pathological aggregation (Dormann et al., 2010; Dewey et al., 2012; Li et al., 2013). However, caution is warranted, as these cell culture experiments used overexpression of TDP-43 and FUS/TLS and do not recapitulate one key feature of TDP-43 and FUS/TLS proteionopathies: concomitant loss of nuclear TDP-43 or FUS/TLS with cytoplasmic inclusions (Mackenzie et al., 2010a).

TDP-43 is transiently lost from neuronal nuclei with concomitant accumulation at injury sites in two in vivo experiments in mice using either axotomy or axonal ligation (Moisse et al., 2009; Sato et al., 2009). Interestingly, mutant TDP-43 showed a delayed response in returning to the nucleus during recovery (Swarup et al., 2012). Since current evidence suggests that at disease end stage TDP-43 and FUS/TLS associate with stress granules in ALS and FTD patients (Dormann et al., 2010; Liu-Yesucevitz et al., 2010), future investigation should now focus on how TDP-43 and FUS/TLS switch from reversible association into irreversible pathological inclusions, what the relationship is between this process and the nuclear clearance of TDP-43 and FUS/TLS, and how the combination of pathological inclusions and loss of nuclear TDP-43 and FUS/TLS drives disease progression.

TDP-43, FUS/TLS, and SMN, a Common Pathogenic Pathway between ALS and Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is a motor neuron disease caused by deficiency in the survival motor neuron (SMN) protein (reviewed in Burghes and Beattie, 2009). SMN is part of a large multiprotein complex that is essential for the biogenesis of spliceosomal-associated small nuclear ribonucleoprotein particles (snRNPs). SMN complexes are found both in the cytoplasm and in nuclear bodies called Gems. Loss of nuclear Gems is a pathological hallmark in SMA. Reduced SMN expression leads to markedly decreased snRNP assembly and reduced snRNA levels in mouse models of SMA and in SMA patients, provoking broad misregulation of RNA splicing.

Recent evidence suggests that perturbation of normal levels of TDP-43 (Shan et al., 2010; Tsuiji et al., 2013) or FUS/TLS (Yamazaki et al., 2012) or expression of ALS-linked mutations in TDP-43 (Yamazaki et al., 2012) and FUS/TLS (Groen et al., 2013; Yamazaki et al., 2012) leads to reduction of nuclear GEM bodies, altered U snRNA expression, and axonal defects, likely through a direct biochemical association between SMN and TDP-43 (Tsuiji et al., 2013) or FUS/TLS (Groen et al., 2013; Yamazaki et al., 2012). Moreover, these SMN deficits are also found in sporadic ALS patients with TDP-43 inclusions (Ishihara et al., 2013; Tsuiji et al., 2013). Taken together, the collective evidence supports convergent pathways of pathogenesis in SMA and ALS, reinforcing the notion that defects in RNA metabolism may be central mechanistic components in motor neuron disease.

Repeat Expansion within C9ORF72

Genome-wide association studies (GWASs) of familial ALS patients in the Finish population, as well as in sporadic ALS, demonstrated the presence of a major ALS locus on chromosome 9p21 (Laaksovirta et al., 2010; Shatunov et al., 2010; Van Deerlin et al., 2010; van Es et al., 2009). The minimal region linking all the families was then narrowed down to a 232 kb interval containing only three protein-coding genes (*MOBKL2B*, *IFNK*, and *C9ORF72*) (Laaksovirta et al., 2010). Rather than the expected amino acid substitutions in a protein coding region, a large GGGGCC hexanucleotide repeat expansion (~700–1,600 copies) within a noncoding region of a gene (*C9ORF72*) was found to be causative (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011).

Hexanucleotide expansion in *C9ORF72* accounts for up to 80% of familial ALS-FTD, 20%–50% of familial ALS, 5%–20% of sporadic ALS, and 10%–30% of FTD, making this repeat expansion the most common cause of ALS and FTD (Boeve et al., 2012; Chiò et al., 2012; Cooper-Knock et al., 2012; Hsiung et al., 2012; Mahoney et al., 2012; Simón-Sánchez et al., 2012; Snowden et al., 2012). Clinically, patients with the *C9ORF72* repeat expansion have been reported to have a higher incidence of bulbar-onset ALS, cognitive impairment with earlier disease onset, and accelerated progression compared with patients without the expansion (Byrne et al., 2012; Chiò et al., 2012; Millecamps et al., 2012; Stewart et al., 2012).

Inclusions containing TDP-43 in brain and spinal cord are prevalent in all patients with the repeat expansion. Additionally, there is the presence of TDP-43-negative cytoplasmic or nuclear inclusions containing either p62/SQSTM1 or ubiquilin-2 or both in the cerebellar granular and molecular layers (Brettschneider et al., 2012). Similarly, TDP-43 pathology is absent from neuronal intranuclear and cytoplasmic inclusions in the pyramidal cell layers of the hippocampus in patients with

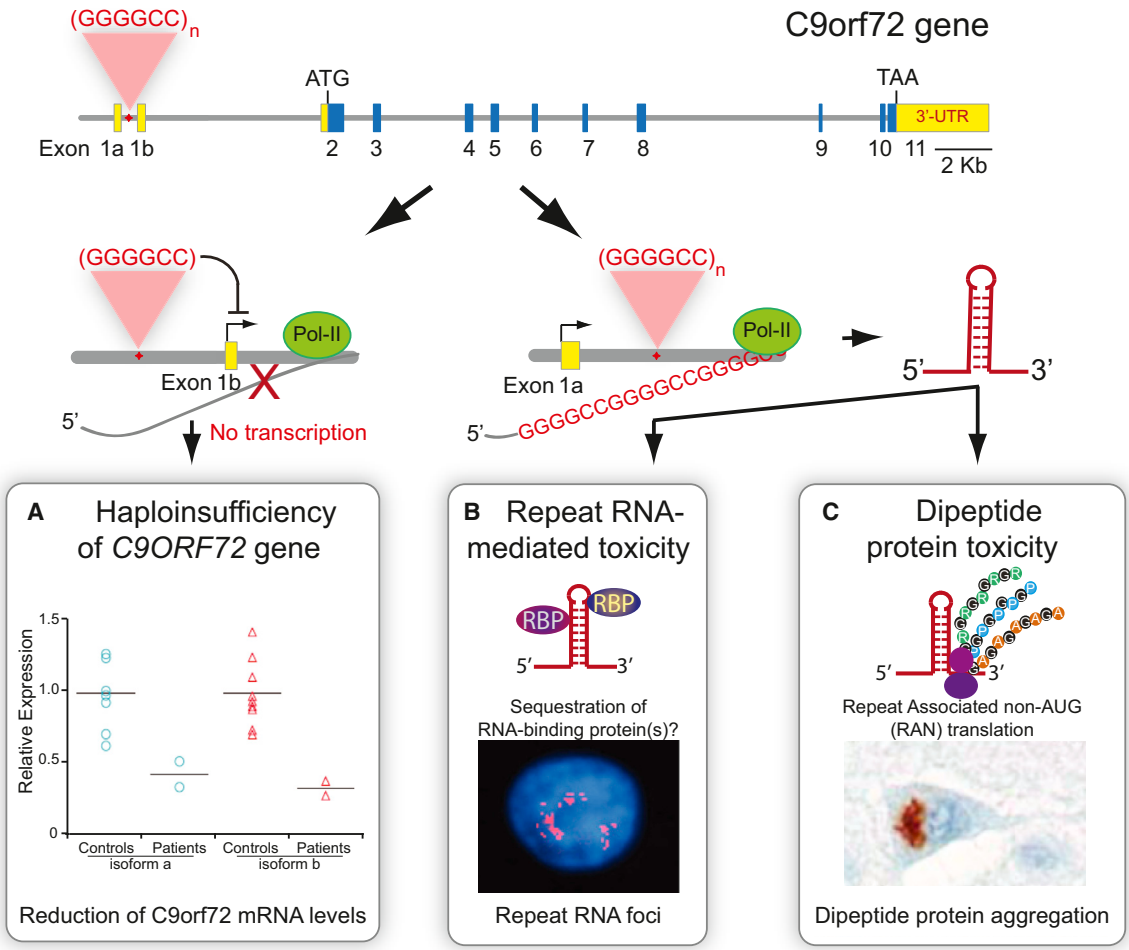

Figure 4. Potential Pathogenic Mechanisms for GGGGCC Repeat Expansion within the C9ORF72 Gene
Top: schematic representation of the human C9ORF72 gene (yellow, UTRs; blue, coding exons). Hexanucleotide (GGGGCC) repeat expansion is located between alternative exons 1a and 1b. At least three mechanisms may contribute to disease pathogenesis: (A) GGGGCC repeat expansions may lead to reduced expression of the allele containing the repeat expansion (haploinsufficiency); (B) RNA foci containing transcribed GGGGCC repeats may sequester RNA-binding protein(s); or (C) repeat associated non-AUG (RAN) translation of GGGGCC repeat-containing RNA produces toxic polydipeptides in each of three reading frames. Data and images reproduced with permission from (A) Gijselinck et al. (2012), (B) DeJesus-Hernandez et al. (2011), and (C) Mori et al. (2013b).

expansion in C9ORF72 (Al-Sarraj et al., 2011; Murray et al., 2011; Troakes et al., 2012), suggesting that additional component(s) other than TDP-43 are present in the inclusions. Cytoplasmic ubiquilin-2-containing inclusions have also been reported in the cerebellar granular and hippocampal molecular layers (Brettschneider et al., 2012).

Pathogenic Mechanisms for the Repeat Expansion in C9ORF72 Gene

The expanded hexanucleotide repeat in the *C9ORF72* gene is reminiscent of multiple prior repeat expansion diseases for which three different prototypes of pathogenic mechanisms have been demonstrated: loss of function of the gene containing the repeat (haploinsufficiency), gain of protein toxicity due to the expression of protein containing the repeat expansion (mutant protein), and gain of RNA toxicity due to the production of RNA containing the repeat (mutant RNA) (La Spada and Taylor, 2010). Additional toxic mechanisms can result from complementary repeat-containing RNA produced by bidirectional transcription (Moseley et al., 2006) or repeat associated non-ATG (RAN) translation (Zu et al., 2011), leading to production, respectively, of potentially toxic RNA and protein species. For *C9ORF72*, because the GGGGCC repeat expansion is located within an alternative non-coding intron 1, the underlying disease pathogenesis may be driven by RNA-mediated or RAN translation-dependent toxicity or haploinsufficiency or any combination of these (Figure 4).

Loss of Function and Gain of Toxicity: Lessons from the Fragile X Locus and Myotonic Dystrophy

The location of the repeat expansion in intron 1 of *C9ORF72* resembles the CGG repeats of the *FMR1* (fragile X mental retardation 1) gene, which, depending on the size of the repeats, yields three different syndromes: fragile X syndrome (>200 repeats), fragile X-associated tremor/ataxia syndrome (50–200 repeats), and premature ovarian insufficiency (50–200 repeats) (Oostra and Willemsen, 2009). Full expansion causes fragile X

syndrome (FXS) from loss of FMR1 gene function mediated by hypermethylation of the adjacent FMR1 promoter region and subsequent transcriptional silencing.

FMR1 carriers with CGG repeats between 50 and 200 develop fragile X-associated tremor/ataxia syndrome (FXTAS) in which the repeats are unmethylated but produce intention tremor, abnormal gait, peripheral neuropathy, and cognitive impairment (Oostra and Willemsen, 2009). In contrast to transcriptional silencing in FXS (Tassone et al., 2000), accumulation of FMR1 mRNA in FXTAS is elevated at least 5-fold, presumably because it is stabilized by binding of hnRNP A2/B1, Pur-α (purine-rich binding protein-α), Sam68, hnRNP-G, along with CUG-binding protein 1 (CUG-BP1) and muscleblind (MBNL1), each of which has been shown either to associate biochemically with the rCGG repeats or colocalize with rCGG RNA foci (Jin et al., 2007; Sellier et al., 2010; Sofola et al., 2007). Furthermore, both Sam68 and hnRNP A2/B1 can be found in the nuclear inclusions of FXTAS patient neurons (Jin et al., 2007; Sellier et al., 2010). While the identities of other RNA-binding proteins potentially trapped in the rCGG foci and the underlying pathogenic mechanisms remain controversial, it is clear that RNA-mediated toxicity is a key component of neurodegeneration in FXTAS. These complications should be borne in mind in considering whether different repeat sizes within the *C9ORF72* gene may provide divergent symptoms/diseases or different severity of phenotypes.

A gain-of-RNA-toxicity mechanism for a repeat expansion disease is best characterized in myotonic dystrophy 1 (DM1), which is caused by up to 2,500 of CTG repeats in the 3′UTR of the myotonic dystrophy protein kinase (DMPK) gene (Lee and Cooper, 2009). Two proteins, CUG-BP1 and muscleblind, were identified to bind to the CUG repeat-containing RNA (Miller et al., 2000; Timchenko et al., 1996). Of these two proteins, only muscleblind shows repeat-length-dependent association and is selectively sequestered into pathogenic RNA foci (Mankodi et al., 2001). Nevertheless, misregulation of both muscleblind and CUG-BP1 play roles in DM1 pathogenesis. Indeed, CUG repeats lead to activation of protein kinase C (PKC), which in turn phosphorylates CUG-BP1, whose phosphorylated form has increased activity from increased protein stability, thereby activating multiple splicing changes toward fetal isoforms (Kuyumcu-Martinez et al., 2007; Roberts et al., 1997).

Evidence for C9ORF72 Haploinsufficiency

The function of the *C9ORF72* gene and its predicted protein product are unknown. Recent bioinfomatical analysis implies a potential involvement of the C9ORF72 protein in membrane trafficking and autophagy (Levine et al., 2013; Zhang et al., 2012), but this remains to be determined. A 50% reduction of mRNA levels corresponding to both short and long mRNA isoforms of *C9ORF72* (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012) has been reported and is consistent with partial or complete silencing of the expanded allele (Figure 4A), although it should be noted that the reduction of the corresponding C9ORF72 proteins has not been demonstrated. Antisense oligonucleotide-mediated reduction of C9ORF72 in zebrafish produces reduced axon lengths of motor neurons and locomotion deficit (Ciura et al., 2013), consistent with the notion that partial loss of the *C9ORF72* gene could contribute to disease pathogenesis.

Evidence for Gain of RNA Toxicity from C9ORF72 Expansion

Intranuclear RNA foci containing the C9ORF72 hexanucleotide repeat have been reported (DeJesus-Hernandez et al., 2011), which may trap one or more RNA-binding proteins, thereby inhibiting their functions, especially in RNA processing (Figure 4B). While two RNA-binding proteins, hnRNP-A3 (Mori et al., 2013a) and Pur-α (Xu et al., 2013), have been reported to bind GGGGCC repeats in vitro and both were reported to be components of p62-positive TDP-43-negative inclusions in C9ORF72 patients, their role in pathogenesis is unproven. Neither has been demonstrated to localize at RNA foci formed by the hexanucleotide repeat and the predicted loss of RNA processing function that would follow from sequestration of hnRNP-A3 and Pur-α has not been demonstrated in cells and tissues expressing the hexanucleotide repeat-containing RNA.

Repeat Non-ATG Translation of C9ORF72 mRNA

Besides the recognized modes of RNA toxicity introduced above, a highly unexpected and potentially toxic mechanism in C9ORF72 has been uncovered: repeat-associated RNA-encoded, non-ATG translation (RAN translation). This phenomenon was originally discovered in spinocerebellar ataxia type 8 (SCA8), a progressive neurodegenerative disease caused by a trinucleotide expansion in the bidirectionally transcribed SCA8 gene (Zu et al., 2011). In one direction, the RNA encoding the ataxin 8 (*ATXN8*) protein contains an in-frame CAG-expansion that is translated into polyglutamine. Surprisingly, this RNA is also translated in an ATG-independent manner in all three reading frames of the CAG repeat both in vitro and in SCA8 human cerebellum.

Following the SCA8 example, two independent studies have now reported translation of the C9ORF72 GGGGCC repeat into polypeptides consisting of repeating di-amino acids: poly-(glycine-alanine, GA), poly-(glycine-proline, GP), and poly-(glycine-arginine, GR) (Figure 4C) that form pathological inclusions in neurons (but not astrocytes) of C9ORF72 patients (Ash et al., 2013; Mori et al., 2013b). Poly-GA is apparently the most prevalent form (Mori et al., 2013b). Moreover, an antisense RNA transcript in C9ORF72 patients has also been reported (Mori et al., 2013b), raising the possibility of two additional dipeptide-repeats (poly-PR and poly-PA), which may also be generated through RAN translation.

More Complexities in Repeat-Mediated Toxicity

If the preceding potential toxicities were not enough, consideration of what is known about SCA8 provides more potential complexities. As mentioned above, the SCA8 locus is bidirectionally transcribed with opposite strand transcription of the CAG repeat, producing a noncoding RNA containing a CUG repeat expansion that sequesters muscleblind, leading to splicing changes similar to those observed in DM1 patients (Daughters et al., 2009; Moseley et al., 2006). Added to potential RAN translation of both CAG and CUG repeats, the pathogenic mechanisms include gain of function at both the protein and RNA levels.

Disruption of Protein Homeostasis in ALS and FTD

Although the chicken-and-egg question persists for whether protein aggregation per se causes or merely reflects a consequence of neurodegenerative diseases, overwhelming evidence supports protein degradation deficits in a wide range of

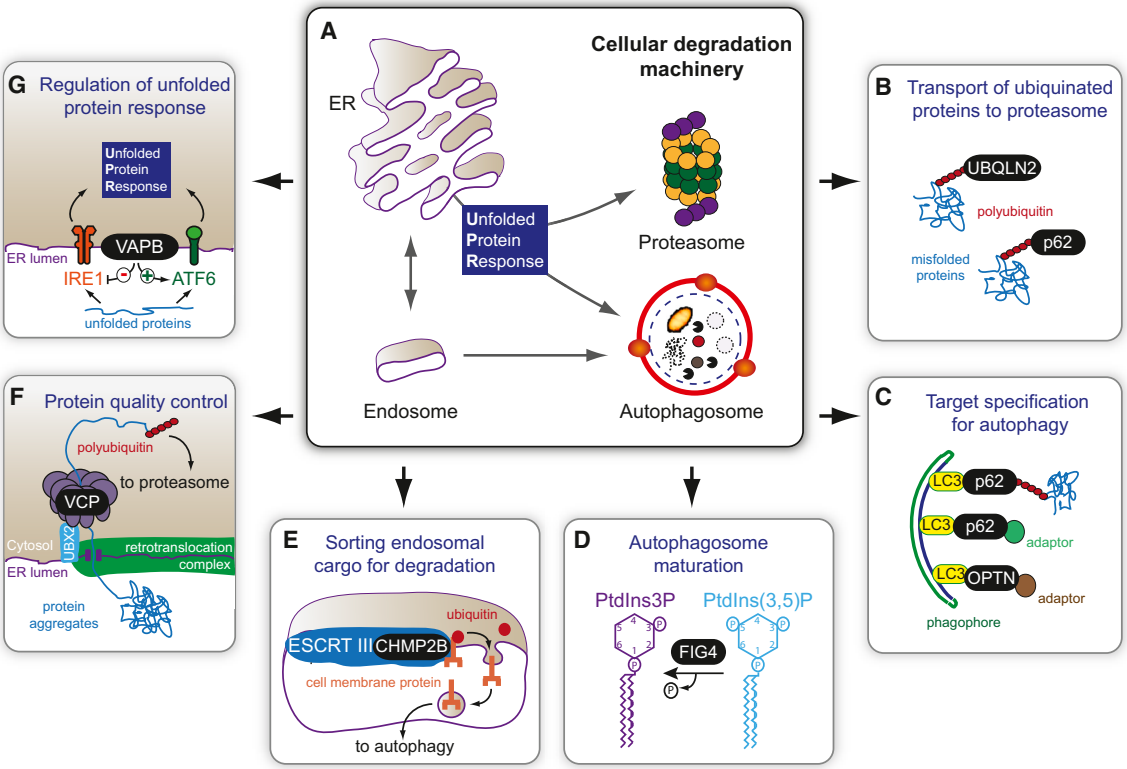

Figure 5. ALS/FTD-Associated Proteins Involved in Protein Homeostasis

(A–C) Schematic representation of major cellular degradation pathways. (A) Many ALS/FTD-linked proteins are involved in protein clearance pathway, including (B) ubiquilin-2 (UBQLN2) and p62/SQSTM1 (which bind to polyubiquitinated proteins and transport them to proteasome for degradation) and (C) p62/SQSTM1 and optineurin (OPTN) (which serve as adaptor proteins to bind simultaneously the substrates that are targeted for autophagy and LC3, a component of autophagosomes).

(D) FIG4 is a lipid phosphatase that converts PI(3,5)P into PI3P. Autophagy is impaired in the neurons and astrocytes missing FIG4, with the disturbance of PIPs expected to disrupt formation or recycling of autolysosomes.

(E) ALS/FTD-linked mutations in CHMP2B, a core component of endosomal sorting complexes (ESCRT) required for transport, disrupt the normal endosome-lysosome-autophagy morphology and function. ESCRT complexes bind to ubiquitinated cargos and initiate clustering of ubiquitinated cargos and membrane invagination. The internalized cargos, which are cell membrane proteins, are transported into autophagosomes for degradation.

(F) VCP interacts with a large number of cofactor proteins that act as ubiquitin adaptors, allowing VCP to interact with a large number of ubiquitinated proteins to enable degradation or recycling, including extraction of misfolded proteins from the ER and subsequently targeting them for degradation.

(G) VAPB is involved in the unfolded protein response (UPR) by modulating activities of the IRE1 and ATF6 arms of the UPR.

disorders through disruption of either of the two major protein clearance pathways: the ubiquitin-proteasome system and autophagy. This is certainly true for ALS/FTD, as demonstrated by identification of ALS- and FTD-linked mutations in genes affecting protein homeostasis, or proteostasis. These genes include ubiquilin-2 (*UBQLN2*), p62/SQSTM1 (sequestosome 1), optineurin (*OPTN*), vasolin-containing protein (*VCP*), charged multivesicular body protein 2B (*CHMP2B*), vesicle-associated membrane protein (VAMP)/synatobrevin-associated protein B (*VAPB*), and *FIG4* (FIG4 homolog, SAC1 lipid phosphatase domain containing protein) (Figure S2). Among these genes, ubiquilin-2, p62, optineurin, and VCP are directly involved in protein degradation, whereas CHMP2B and FIG4 are required for autophagosome maturation (Figure 5).

ALS-FTD mutation in Ubiquilin-2

The ubiquilin protein family brings polyubiquitinated proteins to the proteasome for degradation, and ubiquilins also function in autophagy. The ALS and ALS/dementia-linked mutations initially identified in *UBQLN2* are clustered at or near its proline-rich region, with most altering a conserved proline (P497H, P497S, P506T, P509S, P525S) (Deng et al., 2011; Gellera et al., 2013; Williams et al., 2012). Two additional mutations, S155N and P189T, are located at the N terminus (Daoud et al., 2012). Experiments in cells transfected to express either of two ALS-linked mutations in ubiquilin-2 (R497H and P506T) suggest that that overall protein degradation is impaired (Deng et al., 2011).

Perhaps not surprisingly, colocalization of ubiquilin-2 and ubiquitin in pathological inclusions is seen in patients with *UBQLN2* mutations and these inclusions also contain TDP-43, FUS/TLS, and optineurin (Deng et al., 2011; Williams et al., 2012), suggesting that an impaired protein clearance pathway is a pathogenic mechanism (Figure 5B). Furthermore, ubquilin-2 pathology has been reported in a majority of sporadic ALS (Deng et al., 2011) and hexanucleotide repeat expansion in the

C9ORF72 genes (Brettschneider et al., 2012). Taken together, mutations in ubiquilin-2 provide a mechanistic link of the protein degradation pathway with neurodegeneration.

p62/SQSTM1 Mutations in ALS

Similar to ubiquilin, p62 has been shown to interact with polyubiquitinated proteins (Moscat and Diaz-Meco, 2012) and to interact with LC3, allowing p62 to target polyubiquitinated proteins to the proteasome or autophagy. Therefore, both p62 and ubiquilin-2 link the ubiquitin-proteasome and autophagy pathways (Figures 5B and 5C). Using a candidate gene approach, sequencing of p62/SQSTM1 in familial and sporadic ALS patients revealed several polymorphisms/mutations scattered throughout the coding regions (Fecto et al., 2011; Rubino et al., 2012; Teyssou et al., 2013), accompanied by TDP-43 inclusions (Teyssou et al., 2013). p62-positive inclusions have also been reported in neurons and glia of a wide array of other neurodegenerative diseases (Brettschneider et al., 2012). Although how these ALS-associated variants in p62 contribute to pathogenesis has not been established, autophagy/proteasome disturbance seems likely to play a role.

ALS Mutations in Optineurin

Optineurin (OPTN) is a 577 amino acid multifunctional protein that is able to bind both polyubiquitinated proteins and LC3 (Figure 5C). Indeed, optineurin has been proposed as a receptor for autophagy (Wild et al., 2011). Both nonsense and missense mutations of optineurin have been identified in ALS, accounting ~3% of familial ALS and ~1% of sporadic ALS (Del Bo et al., 2011; Iida et al., 2012a, 2012b; Maruyama et al., 2010; van Blitterswijk et al., 2012). One report has identified OPTN-positive inclusions in patients with OPTN mutation, with TDP-43 inclusions in sporadic ALS and with SOD1-positive inclusions in patients with SOD1 mutations (Maruyama et al., 2010).

ALS- and FTD-Linked Mutations in Vasolin-Containing Protein

Mutations in vasolin-containing protein (VCP) were originally identified as causative of inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMFTD) (Watts et al., 2004) and later in ALS (Johnson et al., 2010). Some of the same mutations have been found for both IBMFTD and ALS (Figure S2). VCP interacts with a large number of ubiquitinated proteins to enable degradation or recycling and functions in multiple protein clearance pathways (Figure 5F), including extracting misfolded proteins from the ER and sorting of endosomal proteins for proper trafficking. Depletion of VCP leads to accumulation of immature autophagosomes, similar to what is observed upon expression of IBMFD-linked mutations (Ju et al., 2009; Tresse et al., 2010), suggesting that VCP is required for proper autophagy. Most intriguingly, TDP-43 is apparently mislocalized to the cytosol upon VCP-mediated autophagic dysfunction (Ju et al., 2009).

FTD- and ALS-Linked Mutations in CHMP2B

Charged multivesicular body protein 2B, or chromatin-modifying protein 2B (CHMP2B) mutations were first identified in FTD (termed FTD-3) (Momeni et al., 2006; van der Zee et al., 2008) and later in ALS (Cox et al., 2010; Parkinson et al., 2006). CHMP2B is a core component of endosomal sorting complexes (reviewed in Raiborg and Stenmark, 2009) (Figure 5E). Multiple studies support mutant CHMP2B-mediated disruption normal endosome-lysosome-autophagy morphology and function (Han et al., 2012a; Urwin et al., 2010; van der Zee et al., 2008). Transgenic mice expressing the intron 5-retention mutant of CHMP2B, but not wild-type CHMP2B, develop progressive neurological deterioration accompanied by axonal pathology and early mortality (Ghazi-Noori et al., 2012). Loss of CHMP2B function, on the other hand, after gene disruption in mice produces no phenotype (Ghazi-Noori et al., 2012).

ALS Mutations in FIG4

FIG4 encodes a 907 amino acid lipid phosphatase that regulates the abundance of phosphatidyl-inositol-3,5-biphosphate ($PI(3,5)P_2$). Recessive mutation in FIG4 causes severe tremor, abnormal gait, degeneration of sensory and motor neurons, and diluted pigmentation in mice. Compound heterozygote mutations, in which a loss-of-function allele combines with a partial loss-of-function mutation, are present in human patients with Charcot-Marie-Tooth disease (CMT4J) (Chow et al., 2007), as are rare, heterozygous variants of FIG4 in ALS (Chow et al., 2009). FIG4 null mice have substantially lowered PI(3,5)P2 levels, which are normally tightly regulated. Not surprisingly, autophagy is impaired in the neurons and astrocytes of mice missing FIG4, with the disturbance of PIPs expected to disrupt formation or recycling of autolysosomes. It is tempting to speculate that ALS-linked variants can tip the balance of phosphoinositide processing and affect autophagic function (Figure 5D).

ALS Mutations in VAPB

Two ALS-linked mutations in the gene encoding VAMP/VAPB have been reported (Figure S2) (Chen et al., 2010; Funke et al., 2010; Millecamps et al., 2010; Nishimura et al., 2004). Expression of either, but not wild-type, in mammalian cell lines produced ER fragmentation and cytoplasmic aggregates of mutant VAPB that also trapped endogenous VAPB (Chen et al., 2010; Kanekura et al., 2006; Nishimura et al., 2004; Teuling et al., 2007). Increased levels of wild-type VAPB elicit the unfolded protein response (UPR) (Figure 5G). Reduction in VAPB attenuates the UPR, as do ALS-linked mutants (Chen et al., 2010; Kanekura et al., 2006), probably by interaction with ATF6, one of the three key molecules in initiating the UPR response (Gkogkas et al., 2008). Transgenic mice expressing wild-type or mutant VAPB (P56S) within the nervous system do not, however, develop overt phenotypes or have reduced survival but do develop cytoplasmic accumulation of ubiquitin, p62, and TDP-43 at 18 months of age (Qiu et al., 2013; Tudor et al., 2010). Nevertheless, along with ALS-, FTD-, and ALS/FTD-linked mutations in ubiquilin-2, p62, optineuron, VCP, CHMP2B, and FIG, the VAPB mutations point to defects in protein clearance as a common component of pathogenesis.

A surprising additional function of VAPB came from study in *Drosophila* of its MSP (major sperm protein) domain (Tsuda et al., 2008). The MSP domain has been reported to be cleaved and secreted, while the ALS-linked P56S mutant abolished the secretion activity and formed ubiquitinated inclusions. Pathogenic mechanisms may involve aberrant Eph signaling. Biochemically, human MSP interacts with EphA4 (Tsuda et al., 2008), a receptor in the ephrin axonal repellent pathway. Intriguingly, EphA4 has been reported to be a genetic modifier for modulating the vulnerability of motor neurons in ALS (Van Hoecke et al., 2012). How the MSP-like fragment is generated in a

mammalian system and whether MSP-EphA4 interaction plays a role in modulating ALS disease course will require further investigation.

SOD1: A Central Component of ALS or an Outlier?

Mutations in the copper/zinc superoxide dismutase 1 (SOD1) gene account for 20% of familial ALS cases (Rosen et al., 1993). Mouse models overexpressing ALS-linked mutations in SOD1 recapitulate most features of ALS pathology, which has led to the discovery of two critical features of SOD1-mediated toxicity: (1) mutant SOD1 causes ALS through a gain of toxic property (or properties), and (2) pathogenesis of the ubiquitously expressed mutant SOD1 is a non-cell-autonomous process. This latter insight was established by gene excision from selected cell types in transgenic mice otherwise expressing mutant SOD1 ubiquitously, an approach that identified disease onset to be driven by mutant synthesized within motor neurons (Boillée et al., 2006; Wang et al., 2009; Yamanaka et al., 2008) and NG2+ oligodendrocyte precursors (Kang et al., 2013), while mutant SOD1 synthesized within two additional glial cell types (astrocytes; Yamanaka et al., 2008; and microglia; Boillée et al., 2006) are primary determinants of accelerated disease progression.

A Crucial Controversy: Is SOD1 a Component of Sporadic Disease?

While ubiquitinated protein aggregates containing SOD1 are a prominent pathological feature in both familial ALS patients with SOD1 mutations and in mice expressing ALS-linked mutations in SOD1 (Bruijn et al., 2004), SOD1-containing inclusions have not been found in most sporadic ALS cases. Nevertheless, early studies hinted that an age-dependent posttranslational and nonmutational modification of SOD1 may be able to change the conformation of wild-type SOD1 into an altered conformation (Bredesen et al., 1997), suggesting that these modified forms of wild-type SOD1 could be contributors to sporadic ALS. The notion that there is a common pathogenic conformation of wild-type and mutant SOD1 has recently made a comeback. Several teams have reported that misfolded SOD1 is present in a portion of sporadic ALS patients (Bosco et al., 2010b; Forsberg et al., 2010; Pokrishevsky et al., 2012). This issue remains highly controversial, with other teams failing to detect misfolded SOD1 in sporadic ALS patients using multiple conformation-specific antibodies (Brotherton et al., 2012; Kerman et al., 2010; Liu et al., 2009).

SOD1 mutant-expressing astrocytes are toxic to cocultured normal motor neurons (Di Giorgio et al., 2007, 2008; Haidet-Phillips et al., 2011; Marchetto et al., 2008; Nagai et al., 2007). Kaspar and colleagues (Haidet-Phillips et al., 2011) reported the very surprising finding that astrocytes derived from autopsy samples from sporadic ALS patients are also toxic to motor neurons. Most provocatively, this team also reported that non-cell-autonomous toxicity to motor neurons from such sporadic ALS-derived astrocytes can be reduced by lowering production of wild-type SOD1, thereby implicating wild-type SOD1 as a contributing factor in sporadic disease. While replication is needed, these results highlight non-cell-autonomous components in ALS pathogenesis and support therapeutic reduction of SOD1 expression in sporadic ALS.

Prion-like Spreading for ALS and FTLD

One of the key features of prion diseases is the conformational conversion of a native state to an infectious, misfolded, and pathological state of the prion protein. The infectious cycle comes from the perpetuating conversion of the normal prion protein into a pathological conformation and spreading to other cells, a process that has now been demonstrated for neurodegenerative diseases such as Alzheimer's and Parkinson's disease (reviewed in Polymenidou and Cleveland, 2012).

Consistent with a prion-like spread, ALS-linked mutant SOD1 can form fibrils (Chattopadhyay et al., 2008) and mutant SOD1 has been shown to possess prion-like aggregation and spreading ability in cultured cells (Grad et al., 2011; Münch et al., 2011), as well as seeding ability using spinal cord homogenate from transgenic animals overexpressing mutant SOD1 (Chia et al., 2010) (Figure 6). Remarkably, increased wild-type SOD1 expression (accompanied by its conversion into an insoluble form) is sufficient to accelerate disease course and shorten survival of SOD1 mutant-expressing mice (Deng et al., 2006), consistent with a prion-like template-dependent aggregation.

Furthermore, both TDP-43 and FUS/TLS contain prion-like domains, which may facilitate seeding and aggregation (Figure 6). Indeed, a recent study reported that intracellular aggregation of TDP-43 can be triggered in cultured cells by transduction of fibrillar aggregates prepared in vitro (Furukawa et al., 2011). More provocatively, insoluble TDP-43 isolated from brains of ALS or FTLD-TDP patients can trigger prion-like templating and aggregation of transfected TDP-43 in cultured cells (Nonaka et al., 2013). In addition, disease-linked mutations in prion-like domains in hnRNP-A2B1 and hnRNP-A1 increase their propensity to form self-seeding fibrils and cross-assemble with wild-type counterparts (Kim et al., 2013). Altogether, along with recognition that the initial symptoms of ALS are typically confined to a particular region, followed by an orderly spread that might be predicted for prion-like propagation, the evidence suggests that a prion-like seeding and spreading mechanism could underlie TDP-43 and FUS/TLS-mediated disease.

Feedforward Loops: Converging Disruptions in RNA and Protein Homeostasis

One of the most devastating features of ALS is the relentless progression and spread of degeneration. We attempt here to provide a molecular basis for this phenomenon. The recent discovery of how RNA granules can form through a low-complexity/prion-like domain in TDP-43, FUS/TLS, and hnRNP A2/B1 (Han et al., 2012b; Kato et al., 2012) has fueled an attractive hypothesis in which prion-like spreading of aggregated SOD1, TDP-43, or FUS/TLS could contribute to ALS pathogenesis (Polymenidou and Cleveland, 2011).

ALS-Linked Mutations in Protein Clearance Pathways Can Lead to TDP-43 Aggregation

Both TDP-43 and FUS/TLS are intrinsically aggregation prone in vitro (Johnson et al., 2009; Sun et al., 2011), which may predispose them to formation of pathological inclusions through their prion-like domains (Kato et al., 2012; Han et al., 2012b; Kim et al., 2013), independent of any proposed progression from an initiating stress granule complex (Dewey et al., 2012). Not surprisingly, both ubiquitin-proteasome and autophagy pathways

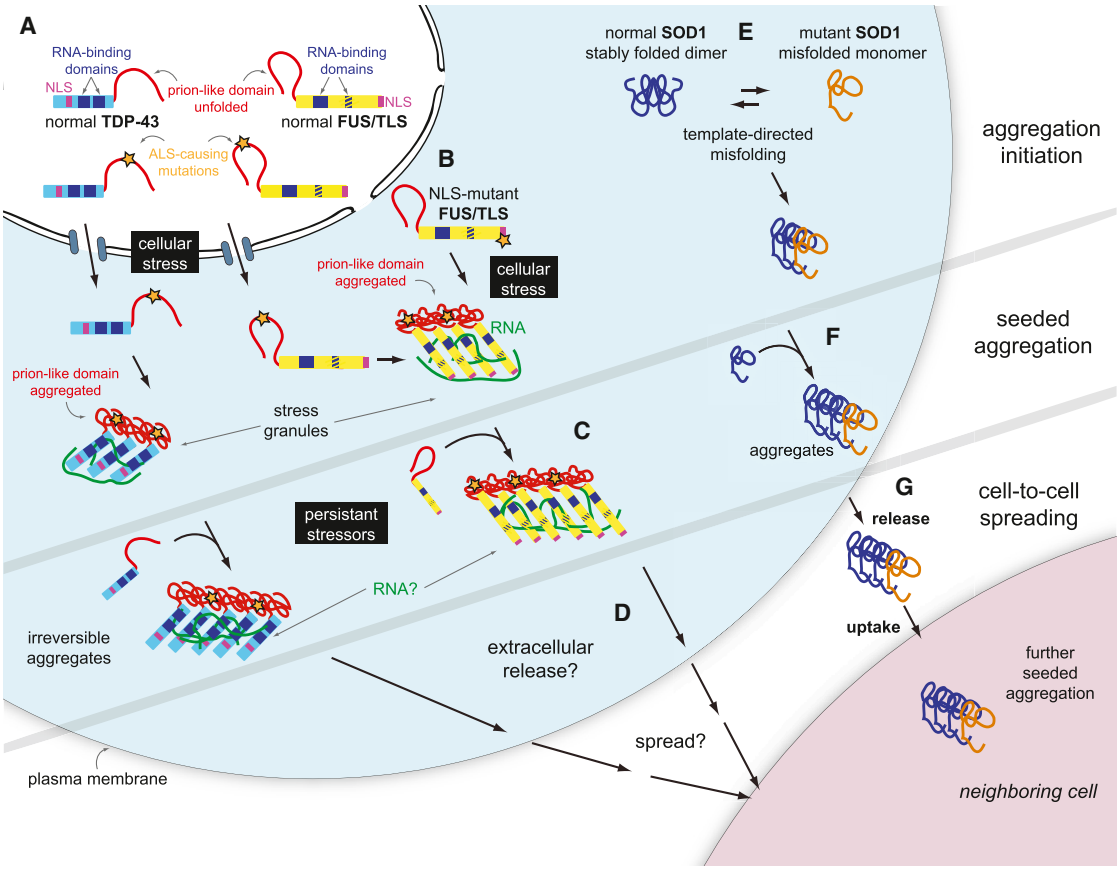

Figure 6. Aggregate Assembly and Propagation in ALS and FTD

Prion-like phenomena in ALS may include SOD1-, TDP-43-, or FUS/TLS-seeded aggregation and cell-to-cell spreading.

(A) TDP-43 and FUS/TLS are both primarily nuclear RNA-binding proteins, whose mutations lead to ALS or FTD. Filled blue boxes on TDP-43 and FUS/TLS molecules indicate RNA-recognition motifs and the striped blue box on FUS/TLS refers to the zinc finger domain that can also bind RNA.

(B) Cellular stress induces TDP-43 and FUS/TLS incorporation into stress granules, which form through the ordered aggregation of several RNA-binding proteins complexed with RNA molecules. This physiologic reaction to cellular stress may be an initial trigger for pathogenic inclusion formation since the increased local protein concentration and RNA scaffolding molecules may facilitate ordered aggregation of TDP-43 and/or FUS/TLS.

(C) Upon chronic cellular stress, defects in stress granule disassembly occurring with aging drive prion-like conformational changes of TDP-43 and FUS/TLS—facilitated by disease-causing mutations in them and stress granule formation—transform into pathogenic self-perpetuating, irreversible aggregates.

(D) Possible cell-to-cell spread (not yet tested for TDP-43 or FUS/TLS) of prion-like aggregates may underlie (or at least contribute to) disease spread from a focal initiation.

(E) Self-perpetuating seeding of misfolded, mutant SOD1 has been reported in cell cultures (Grad et al., 2011; Münch et al., 2011). Acquired damage to wild-type SOD1 may seed similar self-perpetuating aggregates.

(F) Mutant, misfolded SOD1 can induce misfolding of wild-type SOD1 in a template-directed reaction (Chia et al., 2010; Deng et al., 2006), thereby forming a seed of aggregated protein.

(G) SOD1 aggregates transfer from cell to cell to initiate misfolding and aggregation of wild-type or mutant SOD1 in neighboring cells (Münch et al., 2011).

are used for TDP-43 clearance (Brady et al., 2011; Urushitani et al., 2010; Wang et al., 2010). Mutations or disruption of many of ALS-linked genes involved in protein homeostasis pathways (VCP, ubiquilin-2, p62, and CHMP2B) lead to TDP-43 aggregation.

Downregulation of VCP or expression of disease-linked mutations of VCP generate cytosolic TDP-43 aggregations (Gitcho et al., 2009; Ju et al., 2009; Ritson et al., 2010), autophagy defects (Ju et al., 2009), and decreased proteasomal activity (Gitcho et al., 2009). Similarly, reduction of CHMP2B and expression of FTD-linked mutations in CHMP2B inhibit the maturation of autolysosomes, which in turn lead to accumulation of cytosolic TDP-43 aggregates (Filimonenko et al., 2007). Patients with ALS-FTD-linked mutations in ubiliqulin-2, which appear to inhibit proteasome activity, develop TDP-43 proteinopathy (Deng et al., 2011). Ubiquilin-1 interacts with TDP-43 and overexpression of ubiquilin-1 can recruit TDP-43 into cytoplasmic aggregates that colocalize with autophagosomes in cultured cells (Kim et al., 2009). Finally, p62/sequestosome-1 is misaccumulated in both ALS and FTD (Seelaar et al., 2007) along with TDP-43 (Tanji et al., 2012), while increased expression of it reduces TDP-43 aggregates in cultured cells (Brady et al., 2011). Taken together, these findings indicate that ALS/FTD-linked mutations in genes that are involved in protein homeostasis can directly contribute to TDP-43 proteinopathy.

Except for ubquilin-2 mutations (Deng et al., 2011; Williams et al., 2012), inclusion of FUS/TLS has not been reported in response to mutations or disruption of ALS-linked genes involved in the protein homeostasis pathways. However, as described above, one class of ALS-linked mutations disrupts nuclear localization signals, producing higher cytosolic accumulation of FUS/TLS (Dormann et al., 2010; Bosco et al., 2010a). This relocalization of FUS/TLS may be a primary cause for initiating FUS/TLS proteinopathies.

TDP-43 Regulates Expression of ALS-Linked Genes Involved in Protein Clearance

TDP-43 affects levels of RNAs that encode proteins involved in protein homeostasis, including *CHMP2B*, *FIG4*, *OPTN*, *VAPB*, and *VCP* (Polymenidou et al., 2011). Additionally, TDP-43 has been shown to bind the pre-mRNA encoding the autophagy-related 7 (Atg7) protein essential for autophagy, with reduction of TDP-43 downregulating Atg7, thereby impairing autophagy (Bose et al., 2011). It is worth mentioning that mice lacking Atg5 and Atg7 in the nervous system exhibit neurodegeneration (Hara et al., 2006; Komatsu et al., 2006), strongly suggesting—not unexpectedly—that autophagy is essential for normal neuronal function. Altogether, these results suggest an intricate regulatory network in which TDP-43 can affect the expression of the very gene(s) that participate in TDP-43 clearance, providing an additional mechanism of regulating TDP-43 abundance (the other being the autoregulation of TDP-43 by binding to its own mRNA), while TDP-43 also indirectly affects global protein clearance pathways by regulating the expression of key components in autophagy.

Similarly, FUS/TLS binds to the mRNAs encoding optineurin (Lagier-Tourenne et al., 2012; Colombrita et al., 2012), ubiquilin-2 (Lagier-Tourenne et al., 2012; Hoell et al., 2011), VAPB (Lagier-Tourenne et al., 2012; Hoell et al., 2011), and VCP (Lagier-Tourenne et al., 2012; Colombrita et al., 2012; Hoell et al., 2011), although reduction of FUS/TLS in the mouse CNS does not significantly alter their expression levels (Lagier-Tourenne et al., 2012). In a motoneuron-like cell line, FUS/TLS has been argued to be preferentially bound to cytoplasmic mRNAs that are involved in the ubiquitin-proteasome pathway, in particular the cullin-RING E3 ubiquitin ligases (Colombrita et al., 2012). Perhaps most importantly, the endoplasmic reticulum or ubiquitin-proteasome pathways are overrepresented in the mRNA targets bound by ALS-linked mutations in FUS/TLS (Hoell et al., 2011). Altogether, the evidence strongly suggests that, similar to the case for TDP-43, mutation or nuclear loss of function of FUS/TLS affects protein clearance pathways by regulating expression levels of genes in the pathway.

Unifying Underlying Pathogenic Pathways in ALS and FTD

We propose that converging pathogenic mechanisms underlying ALS and FTD are disruption of both RNA and protein homeostasis and disturbed homeostasis that produces a feed-forward loop that drives disease progression (Figure 7). In this model, the initiating event that triggers disease initiation can occur at multiple points in either protein or RNA homeostasis pathways, including genetic mutations that predispose one pathway to be more error prone or other nongenetic factors, such as aging, in which proteostasis decline is well documented.

More provocatively, prion-domain-containing RNA-binding proteins may also be predisposed to self-promoting aggregation and spread, which could explain the seemingly sporadic nature of many instances of both diseases. Subsequent disease progression may be amplified by failure in cross-regulation among multiple proteins/genes, with several ALS-linked genes (including *VCP*, p62/*SQSTM1*, and *CHMP2B*) required for TDP-43 degradation, whereas TDP-43 regulates expression of *VCP* and *CHMP2B*. In addition, not only does TDP-43 bind to its own mRNA, which is essential for its autoregulation, but TDP-43 also binds to several ALS-linked genes involved in RNA homeostasis, including Ang1 (angiogenin), Atxn2 (ataxin-2), and FUS/TLS. Similar mechanisms could exist for FUS/TLS.

Once initiated, errors in RNA and protein homeostasis accumulate, which eventually lead to failure in autoregulation, deregulation of ALS-linked genes, proteotoxic stress, and loss of neuroprotection. The failure to maintain proper protein and RNA homeostasis is highly likely to drive a feedforward cycle, leading to a snowballing effect perturbating many aspects of protein and RNA function. Subsequent propagation and spreading of TDP-43 and FUS/TLS aggregates into neighboring cells could drive spread from a focal initiation site.

Prospects for Therapies in ALS/FTD

Following Jean-Martin Charcot's initial description of ALS, he made the grim statement regarding therapy, "The prognosis, up to the present, is of the gloomiest. There does not exist, so far as I am aware, a single example of a case where, the group of symptoms just described having existed, recovery followed." Sadly, 140 years has passed and ALS remains the same devastating and lethal disease. There is currently only one FDA-approved drug, riluzole, an inhibitor of presynaptic glutamate release, which only extends the survival of the patients for 2–3 months. In the past two decades, many potential therapeutic interventions have been attempted but none have been successful (reviewed in Zinman and Cudkowicz, 2011).

Therapies by Lowering Synthesis of a Toxic Species

For disease from mutant SOD1 (and if wild-type SOD1 is confirmed to be a contributor to sporadic disease), therapy lowering the synthesis of either would be directly on disease mechanism. Indeed, reducing SOD1 expression has been reported to slow disease progression of transgenic mice and rats expressing human mutant SOD1 (Ralph et al., 2005; Raoul et al., 2005; Smith et al., 2006). A further glimmer of hope has emerged from a successful phase I safety trial using antisense oligonucleotides against SOD1 in patients carrying mutant SOD1 (Miller et al., 2013). A similar strategy targeting the potentially toxic RNA species or RAN translation of it can be envisioned for the more frequent instances of disease from hexanucleotide expansion in *C9ORF72*.

Therapy Design by Improving Protein Homeostasis

Several lines of evidence indicate that broad defects in protein homeostasis may contribute to ALS pathogenesis: (1) all ALS patients have one of the following protein inclusions in affected motor neurons: TDP-43, FUS/TLS, or SOD1; (2) ALS-linked mutations are identified in several genes involved in ER stress,

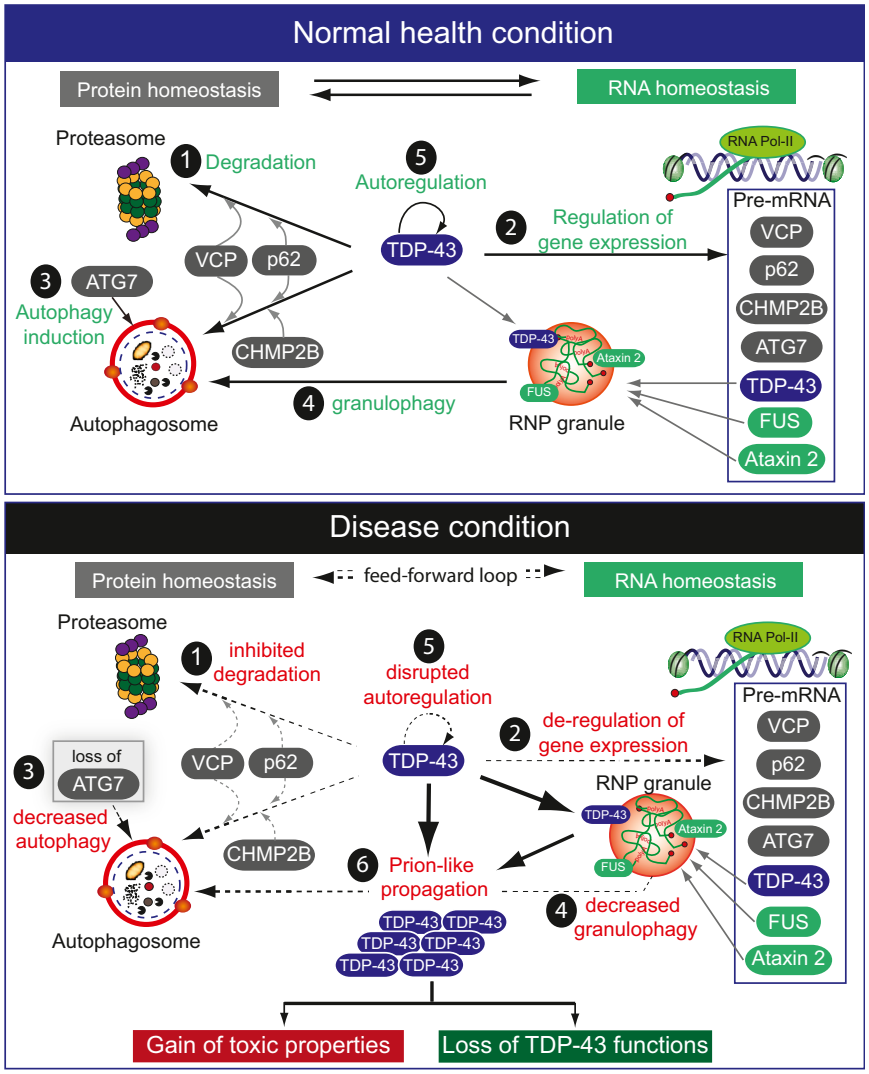

Figure 7. Molecular Interplay of TDP-43 with Other ALS- and FTD-Linked Genes in Protein and RNA Homeostasis

Proposed pathogenic mechanisms in TDP-43-mediated neurodegeneration converge onto disruption of protein and RNA homeostasis. Proteins involved in protein and RNA homeostasis are labeled as gray and green, respectively. Top: normal functions of ALS/FTD-linked proteins. (1) Three ALS-linked genes (VCP, p62, and CHMP2B) are required for proper turnover of TDP-43, which is degraded both by the proteasome and by autophagy. (2) TDP-43 regulates the expression of the same ALS-linked genes that are required for its own degradation as well as (3) a key autophagy induction protein, ATG7. TDP-43 also regulates two other RNA-binding proteins linked to ALS (FUS/TLS and Ataxin-2). TDP-43, FUS/TLS, and Ataxin-2 form RNA protein granules that are degraded through (4) autophagy/granulophagy. Thus, TDP-43 governs both protein and RNA homeostasis and (5) its own level is tightly maintained. Bottom: disrupted protein and RNA homeostasis that fuels a feedforward loop driving disease progression. An initiating event that triggers disease initiation can occur at multiple points in either protein or RNA homeostasis pathways, including genetic mutations that predispose one pathway to be more error prone or other nongenetic factors, such as aging. The well-documented (1) decline in proteostasis during aging may lead to elevated accumulation of TDP-43. Subsequently, (2) the genes that are controlled by TDP-43 become deregulated, including (3) loss of expression of ATG7, which in turn reduces (4) autophagy (and granulophagy). The net result of this produces (5) disrupted autoregulation of TDP-43 with an increased cytoplasmic concentration of TDP-43, which provokes (6) prion-like templating of it, followed by propagation and spread. These gain-of-toxic properties induce overproduction of "nonfunctional prion-like" TDP-43 that leads to further loss of TDP-43 function. Similar scenarios could operate for prion-like domain-containing RNA-binding proteins, such as FUS/TLS and hnRNP A2/B1.

autophagy, and the ubiquitin-proteasome pathway; (3) ALS-linked mutations in ubiquilin-2, CHMP2B, and VCP can lead to TDP-43 aggregation; (4) dysfunctions in ERAD and autophagy are observed in mouse models expressing mutant SOD1; and (5) autophagy appears to be activated and upregulated in motor neurons of sporadic ALS patients.

It is not clear how a decline in general protein degradation machinery might cause aggregation of specific proteins in different neurodegenerative diseases. However, it is conceivable that increasing (or delaying age-dependent decline in) proteostasis could, in principle, prevent or slow down the formation of protein inclusions—or at least accumulation of some or all of the toxic protein species. Initial hints that this approach could be beneficial came from report of modest delay in disease progression following treatment of a very small number of mice with arimoclomol, an inducer of heat shock proteins HSP70 and HSP90 (Kieran et al., 2004). Phase 2/3 clinical trials are currently underway for this approach. Dampening the UPR by deleting a downstream X-box-binding protein (XBP-1) was reported to provide a modest survival benefit (~20 days) to a small cohort (n = 7) of SOD1^{G86R} mice, but the apparent benefit was disappointingly found only in female mice (Hetz et al., 2009). Finally, pharmacological activation of autophagy was reported in another small cohort of mice (n = 10 per drug treatment) to improve cognitive and motor phenotype in male mice overexpressing wild-type TDP-43 (Wang et al., 2012).

Independent replications of the above experiments with larger cohorts that are powered to provide statistical significance—and extended to multiple ALS/FTD mouse models—are now needed to validate the therapeutic potential of these approaches. Moreover, enthusiasm for the rationale of autophagy induction must be tempered by recognition that simply activating the autophagy pathway may cause cytosolic depletion of essential organelles (reviewed in Wong and Cuervo, 2010), and it should be recognized that autophagy is a double-edged sword.

Concluding Remarks

With recent advances in identifying major common genetic causes and the identities of major components in the pathological aggregates for ALS and FTD, perturbation of both RNA and protein homeostasis is a convergent molecular feature with a probable feedforward loop driving the failure in maintaining RNA and protein homeostasis as a central underlying mechanism for the relentless deterioration of neurons. There is probably no silver bullet for curing all sporadic cases. However, with knowledge of genetic causes and molecular players, it is the most exciting time for discovery in ALS and FTD. Much remains still to be learned, bearing in mind Charcot's charge from 140 years ago, "Let us keep searching. It is indeed the best method of finding and perhaps thanks to our efforts, the verdict we will give such a patient (with ALS) tomorrow will not be the same we must give this man today."

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.07.033.

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