

Biology and Pathobiology of TDP-43 and Emergent Therapeutic Strategies

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Cytoplasmic TDP-43 mislocalization and aggregation is a pathological hallmark of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. TDP-43 is an RNA-binding protein (RBP) with a prion-like domain (PrLD) that promotes TDP-43 misfolding. PrLDs possess compositional similarity to canonical prion domains of various yeast proteins, including Sup35. Strikingly, disease-causing TDP-43 mutations reside almost exclusively in the PrLD and can enhance TDP-43 misfolding and toxicity. Another ~70 human RBPs harbor PrLDs, including FUS, TAF15, EWSR1, hnRNPA1, and hnRNPA2, which have surfaced in the etiology of neurodegenerative diseases. Importantly, PrLDs enable RBP function and mediate phase transitions that partition functional ribonucleoprotein compartments. This PrLD activity, however, renders RBPs prone to populating deleterious oligomers or self-templating fibrils that might spread disease, and disease-linked PrLD mutations can exacerbate this risk. Several strategies have emerged to counter TDP-43 proteinopathies, including engineering enhanced protein disaggregases based on Hsp104.

One of the greatest biomedical challenges of our era lies in the daunting reality that there continues to be no effective therapies for several ineluctably fatal and increasingly common neurodegenerative disorders connected with protein misfolding, soluble toxic oligomers, and aberrant protein aggregation (Cushman et al. 2010; Eisenberg and Jucker 2012; Prusiner 2013). One of these debilitating neurodegenerative disorders, amyotrophic lateral sclerosis (ALS), is the most common adult motor neuron disease, afflicting ~2 individuals per 100,000, with typical onset between 50–60 years of age (Robberecht and Philips 2013). Upon progression, ALS is distinguished by an unrelenting

devastation of upper and lower motor neurons (Robberecht and Philips 2013). This neurodegeneration leads to progressive weakness, muscular wasting, and spasticity, which culminates in paralysis, denervation of respiratory muscles, and typically death within ~3–5 years (Robberecht and Philips 2013). There are no effective therapies for ALS, although riluzole can sometimes extend survival by ~2–3 months (Bensimon et al. 1994). Riluzole efficacy is limited and is further compounded by poor central nervous system bioavailability (Jablonski et al. 2014). The lack of treatment options is unacceptable, and we are in urgent need of effective therapeutics (Zinman and Cudkowicz 2011).

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A neuropathological hallmark of ALS is the accumulation of nonamyloid, ubiquitin-positive inclusions in the cytoplasm of degenerating motor neurons and glia (Gros-Louis et al. 2006; Neumann et al. 2006; Pasinelli and Brown 2006; Kwong et al. 2008). The identity of the protein found in these inclusions varies depending on the specific form of ALS. ALS is mostly a sporadic disease (sALS), but ~10% of ALS cases are familial (fALS) (Rowland and Shneider 2001; Renton et al. 2014). Mutations in SOD1, the gene encoding Cu/Zn superoxide dismutase (Gros-Louis et al. 2006; Pasinelli and Brown 2006), have been identified in ~20% of fALS cases for an overall incidence of ~2% (Valentine and Hart 2003). In these cases, SOD1 is found in protein inclusions in afflicted neurons (Rotunno and Bosco 2013). However, whether wild-type (WT) SOD1 misfolds and contributes to sALS remains uncertain (Rotunno and Bosco 2013). Regardless, neurons of ALS patients without SOD1 mutations contain numerous ubiquitin-positive inclusions that are SOD1 negative (Mackenzie et al. 2007), suggesting the presence of additional pathological proteins.

It is now clear that in the vast majority of sALS and fALS cases (~97%) these protein inclusions are comprised of the RNA-binding protein (RBP) TDP-43 (Arai et al. 2006; Neumann et al. 2006; Davidson et al. 2007; Seelaar et al. 2007; Tan et al. 2007; Lagier-Tourenne and Cleveland 2009; Lagier-Tourenne et al. 2010; Ling et al. 2013). Indeed, mutations in eight different genes connected to ALS, *TARDP*, *C9ORF72*, *PGRN*, *VCP*, *UBQLN2*, *ANG*, *OPTN*, and *NIPA1*, result in TDP-43 proteinopathy (Lee et al. 2012; Robberecht and Philips 2013; Renton et al. 2014). However, in some ALS cases, neither TDP-43 nor SOD1 aggregates, but instead another RBP, FUS, forms cytoplasmic aggregates. TDP-43 and FUS pathology are mutually exclusive (Neumann et al. 2009; Vance et al. 2009; Hewitt et al. 2010; Mackenzie et al. 2010; Seelaar et al. 2010). Thus, two ubiquitously expressed and predominantly nuclear RBPs, TDP-43 and FUS, have risen to prominence in ALS (Neumann et al. 2006; Kwiatkowski et al. 2009; Lagier-Tourenne and Cleveland 2009; Vance et al. 2009; Lagier-Tou-

renne et al. 2010; Da Cruz and Cleveland 2011; Robberecht and Philips 2013). Importantly, mutations in the genes that encode TDP-43 and FUS cause ~10% of fALS cases (Da Cruz and Cleveland 2011; Renton et al. 2014). TDP-43 and FUS are of particular interest because they are aggregated in the cytoplasm of sALS cases (Neumann et al. 2006; Pesiridis et al. 2009; Deng et al. 2010; Hewitt et al. 2010; Rademakers et al. 2010; Fujita et al. 2011; see also Mackenzie and Neumann 2016; Nonaka and Hasegawa 2016).

It is important to note that TDP-43, FUS, and SOD1 are not the only proteins that misfold and aggregate in ALS. Depending on the precise form of the disease, in addition to TDP-43 pathology, other RBPs can also form inclusions, including TAF15, EWSR1, hnRNP1, hnRNP2, and hnRNP3 (Couthouis et al. 2011, 2012; Kim et al. 2013; Mori et al. 2013b). Moreover, the most common genetic cause of ALS is a massive expansion of the GGGGCC hexanucleotide repeat in the first intron of the *C9ORF72* gene (DeJesus-Hernandez et al. 2011; Renton et al. 2011, 2014). The first intron of the *C9ORF72* gene contains a polymorphic hexanucleotide repeat, GGGGCC (DeJesus-Hernandez et al. 2011; Renton et al. 2011). The repeat tract length in unaffected individuals (although variable) is typically between ~5 and 10 repeats and is almost always fewer than ~23 repeats (DeJesus-Hernandez et al. 2011; Renton et al. 2011; Rohrer et al. 2015). In *C9ORF72*-ALS cases, the hexanucleotide repeat tract is expanded to hundreds or even thousands of repeats, which cause impairments in nucleocytoplasmic transport (Acharya et al. 2006; DeJesus-Hernandez et al. 2011; Renton et al. 2011, 2014; Fox and Tibbetts 2015; Freibaum et al. 2015; Jovicic et al. 2015; Rohrer et al. 2015; Zhang et al. 2015). Intriguingly, the pathogenic GGGGCC repeat expansion can be translated, even in the absence of an ATG start codon and even though it is located in a noncoding region of *C9ORF72* (Mori et al. 2013a,c). This unconventional repeat-associated non-ATG translation (Zu et al. 2011) occurs in all sense and antisense reading frames, producing polymers of the predicted dipeptides (sense: GGG GCC = Gly-Ala; GGG CCG =

Gly-Pro;GGCCGG = Gly-Arg; antisense: CCC CGG = Pro-Arg; CCC GGC = Pro-Gly; CCG GCC = Pro-Ala) (Mackenzie et al. 2013; Mori et al. 2013a,c; Zu et al. 2013). Remarkably, these various dipeptide repeat proteins form cytoplasmic inclusions in neurons of ALS and frontotemporal lobar degeneration (FTLD) patients with the *C9ORF72* GGGGCC repeat expansion mutation, but not in ALS cases without the *C9ORF72* GGGGCC repeat expansion (Mackenzie et al. 2013; Mori et al. 2013a,c; Zu et al. 2013). Moreover, poly-GA, poly-GR, and poly-PR are highly toxic in model systems independent of the GGGGCC repeat expansion, and poly-GR and poly-PR can directly impair nucleocytoplasmic transport (Kwon et al. 2014; May et al. 2014; Mizielinska et al. 2014; Paul and Gitler 2014; Wen et al. 2014; Jovicic et al. 2015; Tao et al. 2015; Tran et al. 2015). Thus, multiple proteins are misfolded and aggregated in ALS, and the precise set of affected proteins depends on the underlying cause of disease.

Just as other proteins can be aggregated in ALS, TDP-43 misfolds and aggregates in other neurodegenerative diseases as well. Indeed, pathology and genetics have also connected TDP-43 misfolding to a subset of FTL cases (FTLD-TDP) (Arai et al. 2006; Neumann et al. 2006; Mackenzie et al. 2010; Robberecht and Philips 2013). FTL is a devastating neurodegenerative disorder characterized by progressive loss of neurons in the frontal and temporal lobes (Irwin et al. 2015). This neurodegeneration predominantly affects behavior, social awareness, and language and is marked by a severe deterioration in functioning and typically death ~8 years after the onset of symptoms (Irwin et al. 2015). As with ALS, in FTL-TDP, TDP-43 is mislocalized to cytoplasmic inclusions and depleted from the nucleus in afflicted neurons (Arai et al. 2006; Neumann et al. 2009; Mackenzie et al. 2010; Irwin et al. 2015). Interestingly, sometimes neurons in the prefrontal and temporal cortex are also affected in ALS (~15% of cases), and, likewise, FTL can present with ALS (~15% of cases) (Robberecht and Philips 2013). Thus, ALS and FTL appear to represent opposite ends of the clinical spectrum of a single disease (Geser et al. 2009; Robberecht and

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Philips 2013). Prominent TDP-43 cytoplasmic mislocalization and aggregation is also evident in Perry syndrome, Alexander disease, and multisystem proteinopathy (MSP), also known as inclusion-body myopathy with Paget's disease of bone and frontotemporal dementia with ALS (IBMPFD/ALS) (Weihl et al. 2008; Chen-Plotkin et al. 2010; Benatar et al. 2013; Kim et al. 2013; Walker et al. 2014). Remarkably, TDP-43 pathology is a secondary feature of several other neurodegenerative disorders, including Alzheimer's disease (AD), Lewy body (LB)-related diseases (e.g., Parkinson's disease), and Huntington's disease (Amador-Ortiz et al. 2007; Nakashima-Yasuda et al. 2007; Schwab et al. 2008; Uryu et al. 2008; Chen-Plotkin et al. 2010; Josephs et al. 2014a, 2016). TDP-43 lesions appear to worsen several clinical and pathological parameters observed in AD and LB-related disorders, indicating combinatorial effects (Nakashima-Yasuda et al. 2007; Josephs et al. 2014b; Jung et al. 2014). Collectively, these advances indicate that TDP-43 misfolding likely contributes to a broad range of neurodegenerative conditions and could be an important therapeutic target. Here, we review the biology and pathobiology of TDP-43 and consider possible therapeutic strategies to mitigate TDP-43 misfolding and toxicity in disease.

TDP-43 PERFORMS DIVERSE FUNCTIONS IN THE NUCLEUS AND CYTOPLASM

TDP-43 is a 414 amino acid RBP bearing two RNA-recognition motifs (RRMs): RRM1 (amino acids 105–169) and RRM2 (amino acids 193–253) (Fig. 1A) (Buratti and Baralle 2001). TDP-43 displays specificity for UG-rich RNA and TG-rich DNA (Buratti and Baralle 2001; Polymenidou et al. 2011; Lukavsky et al. 2013; Qin et al. 2014). RRM2 contains a nuclear export sequence (NES; amino acids 239–250) (Fig. 1A) (Winton et al. 2008). The RRM2 is flanked on the N-terminal side by an N-terminal domain (NTD; amino acids 1–78) that adopts a ubiquitin-like fold and a canonical nuclear localization sequence (NLS; amino acids 82–98), and they are flanked on the C-terminal side by a low complexity prion-like domain (PrLD; amino

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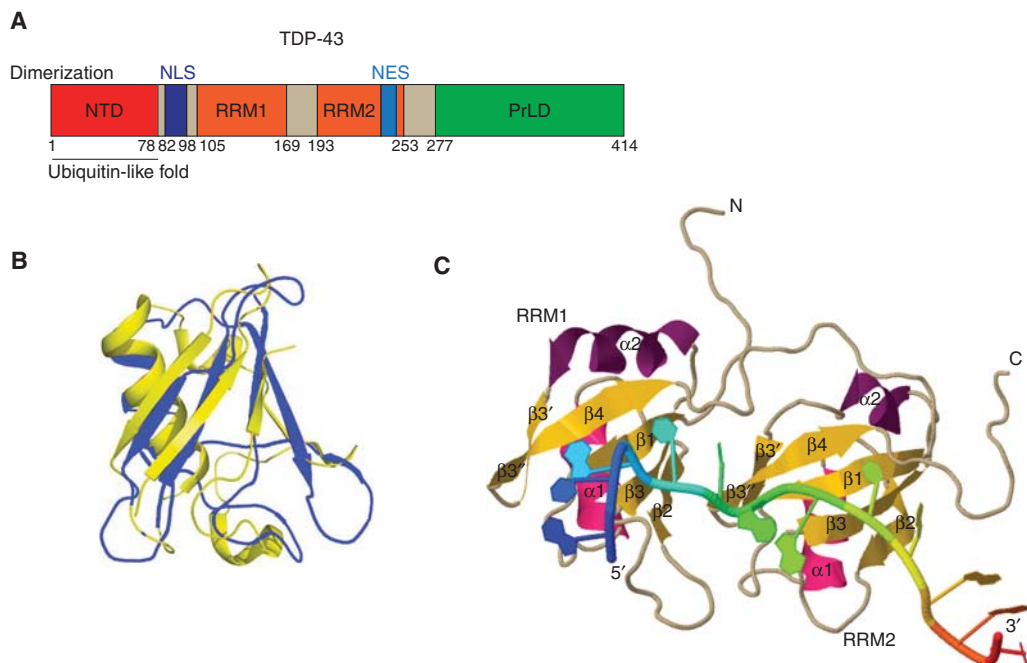


Figure 1. TDP-43 structure. (A) Domain architecture of TDP-43. Domain boundaries are numbered according to the full-length protein sequence. (B) Overlay of the TDP-43 N-terminal domain (NTD) structure (blue) and ubiquitin (yellow; PDB ID code 3EHV) (Qin et al. 2014). (C) Ribbon representation of the structure of the TDP-43 RNA-recognition motif (RRM) construct (amino acids 102–269) in complex with AUG12 RNA (PDB ID code 4BS2) (Lukavsky et al. 2013).

acids 277–414) (Fig. 1A) (Winton et al. 2008; Cushman et al. 2010; Fuentealba et al. 2010; Couthouis et al. 2011; King et al. 2012; Li et al. 2013; Qin et al. 2014; Mompean et al. 2016).

TDP-43 is an essential gene in mammals, zebrafish, and flies (but curiously is not essential in *Caenorhabditis elegans*). It is broadly expressed and, under normal conditions, resides predominantly in the nucleus but shuttles to and from the cytoplasm (Ayala et al. 2008; Winton et al. 2008; Feiguin et al. 2009; Lu et al. 2009; Chiang et al. 2010; Kraemer et al. 2010; Sephton et al. 2010; Zhang et al. 2012; Schmid et al. 2013). TDP-43 expression level is very tightly regulated (Ayala et al. 2011; Polymenidou et al. 2011). Indeed, overexpression of TDP-43 is connected with FTL (Gitcho et al. 2009) and is detrimental in diverse model systems (Johnson et al. 2008; Ash et al. 2010; Elden et al. 2010; Tsai et al. 2010; Xu et al. 2010; Estes et al. 2011; Igaz et al. 2011; Zhang et al. 2011). TDP-43

directly regulates its own expression by binding to the 3'UTR of its own mRNA and promoting its degradation (Ayala et al. 2011; Polymenidou et al. 2011; Avendano-Vazquez et al. 2012).

In the nucleus, TDP-43 performs several important functions (Ling et al. 2013). It engages promoter regions of genes and can repress transcription (Ou et al. 1995; Acharya et al. 2006; Lalmansingh et al. 2011). As well as directly binding DNA, TDP-43 physically interacts with various proteins involved in transcription (e.g., methyl-CpG-binding protein 2), although proteomic analysis suggests that TDP-43 predominantly interacts with proteins involved in splicing and translation (Freibaum et al. 2010; Sephton et al. 2011). However, the precise set of genes that are regulated at the transcriptional level by TDP-43 remains unclear. At the RNA level, TDP-43 performs diverse functions in the nucleus, which include splicing and inhibition of exon recognition,

long intron binding and stabilization, micro-RNA biogenesis, co-transcriptional limitation of double-stranded RNA formation, inhibition of specific RNA editing events, and binding long noncoding RNA (Buratti and Baralle 2001; Buratti et al. 2004; Ayala et al. 2006; Polymenidou et al. 2011; Kawahara and Mieda-Sato 2012; Lagier-Tourenne et al. 2012; Ling et al. 2013; Saldi et al. 2014; Ling et al. 2015). TDP-43 also performs critical RNA-binding modalities in the cytoplasm related to RNA transport, translation, and stress-granule formation (Liu-Yesucevitz et al. 2010; Nishimoto et al. 2010; McDonald et al. 2011; Li et al. 2013; Ling et al. 2013; Alami et al. 2014; Coyne et al. 2014; Liu-Yesucevitz et al. 2014). These collective nuclear and cytoplasmic functions are facilitated by the RNA-binding activity of TDP-43 coupled with interactions with numerous hnRNPs (e.g., hnRNPA1 and A2), microprocessor proteins (e.g., dicer and drosha), and splicing factors (e.g., PSE, splicing factor 3a, PTBP2) (Buratti et al. 2005; D'Ambrogio et al. 2009; Freibaum et al. 2010; Ling et al. 2010; Sephton et al. 2011; Kawahara and Mieda-Sato 2012). It is estimated that TDP-43 binds to >6000 RNA targets in the brain, thereby impacting ~30% of the transcriptome (Polymenidou et al. 2011; Tollervey et al. 2011). In the brain, TDP-43 affects the splicing of ~950 mRNAs and engages the 3'UTR of >1000 mRNAs (Polymenidou et al. 2011; Tollervey et al. 2011). TDP-43 also engages and stabilizes very long introns in various pre-mRNAs and is estimated to affect the levels of >600 mRNAs in the mammalian nervous system (Polymenidou et al. 2011; Tollervey et al. 2011).

TDP-43 STRUCTURE AND FUNCTION

The structure of full-length TDP-43 remains unknown, and so precisely how TDP-43 accomplishes these diverse tasks remains uncertain as well. Nonetheless, biochemical and functional studies have yielded important insights. Several lines of evidence suggest that TDP-43 must form homodimers for optimal functionality, and dimerization appears to be mediated by the first 10 amino acids of the TDP-43 NTD

(Fig. 1A) (Johnson et al. 2009; Shiina et al. 2010; Wang et al. 2013; Zhang et al. 2013; Kuo et al. 2014; Sun et al. 2014). Further domain requirements for TDP-43 activity have been elucidated in various functional studies. For example, considerable interplay between RRM1 and RRM2 contributes to complex formation with RNA (Buratti and Baralle 2001; Lukavsky et al. 2013). Highly conserved phenylalanines in RRM1 (F147 and F149) and RRM2 (F194, F229, and F231) engage RNA directly, although RRM2 makes a weaker contribution to the overall TDP-43 RNA-binding affinity (Buratti and Baralle 2001; Lukavsky et al. 2013). In contrast, the C-terminal PrLD is not typically required for TDP-43 to bind RNA or DNA, although in isolation the TDP-43 PrLD can directly bind single-stranded DNA (Lim et al. 2016). Nonetheless, the PrLD is critical for TDP-43 activity in alternative splicing of some mRNAs and mediates protein–protein interactions with other hnRNPs, including hnRNPA1, A2, and FUS, as well as components of the dicer and drosha complexes (Ayala et al. 2005; Buratti et al. 2005; D'Ambrogio et al. 2009; Kim et al. 2010; Kawahara and Mieda-Sato 2012). However, alternative splicing of other mRNAs depends strictly on RRM1, whereas RRM2 and the PrLD are less important (Fiesel et al. 2012). Likewise, TDP-43 activity in transcriptional repression can be mediated solely by RRM1 (Lalmansingh et al. 2011), whereas TDP-43 recruitment to stress granules requires RRM1 and the PrLD (Bentmann et al. 2012). Thus, a picture is gradually emerging in which the complex multidomain architecture of TDP-43 is deployed in various ways to enable specific functions.

Structural studies of isolated TDP-43 domains have also started to yield key insights. For example, initial studies suggested that the isolated NTD (amino acids 1–78) adopts a novel ubiquitin-like fold (Fig. 1B), even though the primary sequence bears little resemblance to ubiquitin (Qin et al. 2014). The significance of this putative ubiquitin-like fold in the TDP-43 NTD remains unclear, but ubiquitin-like domains in other proteins can mediate diverse functions, including direct binding to the proteasome (Madsen et al. 2007; Grabbe and Dikic

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2009). The TDP-43 NTD engages single-stranded TG-rich DNA in a sequence-specific manner, and addition of UG-rich RNA may induce aggregation of the TDP-43 NTD (Qin et al. 2014). The role of the potential ubiquitin-like fold in TDP-43 function and disease warrants further study and connects TDP-43 to another ALS disease protein, ubiquilin-2, which also harbors a ubiquitin-like domain (Hanson et al. 2010; Deng et al. 2011).

More recent studies, however, suggest that the TDP-43 NTD contains additional structural elements, including two β -strands that form a β -hairpin, which is not found in the ubiquitin fold (Mompean et al. 2016). Thus, the TDP-43 NTD may more closely resemble the C-terminal Dix domain of Axin 1 (Mompean et al. 2016). This finding suggests that the TDP-43 NTD may be more likely to bind cationic protein structures rather than nucleic acids (Mompean et al. 2016).

The structure of a TDP-43 construct comprising both RRM1 (amino acids 102–269) bound to UG-rich RNA oligonucleotide, AUG12 (5'-GUGUGAAUGAAU-3'), revealed that RRM1 and RRM2 adopt a canonical RRM fold ($\beta 1\alpha 1\beta 2\beta 3\alpha 2\beta 4$) with an additional β -hairpin ($\beta 3'\beta 3''$) inserted between $\alpha 2$ and $\beta 4$ (Fig. 1C) (Lukavsky et al. 2013). The β -hairpin insertion creates an expanded β -sheet surface accessible for RNA binding (Fig. 1C) (Lukavsky et al. 2013). An extended, positively charged groove on the β -sheet surface accommodates the AUG12 RNA in a single-stranded conformation in which 10 of the 12 nucleotides directly contact the protein (Lukavsky et al. 2013). Importantly, six of these G or U nucleotides are recognized sequence specifically, which explains the specificity of TDP-43 for UG-rich RNA (Lukavsky et al. 2013). The RNA is bound in a 5' to 3' direction from RRM1 to RRM2 (Fig. 1C), in contrast to several other tandem RRM proteins that engage RNA in a 5' to 3' direction from RRM2 to RRM1 (Lukavsky et al. 2013). A central G nucleotide engages both RRM1 and RRM2 and thereby stabilizes a specific tandem RRM arrangement, which enables TDP-43 to bind RNA and promote splicing activity (Lukavsky et al. 2013). Indeed, splicing repression

by TDP-43 is mediated by sequence specificity for UG-rich RNA encoded by both RRM1, high-affinity RNA binding by RRM1, and exact spatial positioning of the RRM1 relative to one another (Lukavsky et al. 2013).

The C-terminal, low complexity PrLD of TDP-43 (amino acids 277–414) is enriched in uncharged polar amino acids and glycine and contains two tracts (amino acids 277–293 and 346–378) that are predicted to be intrinsically unfolded (Cushman et al. 2010; King et al. 2012). The unusual amino acid composition of the PrLD resembles that of canonical prion domains of various yeast proteins, including Sup35 and Mot3 (Cushman et al. 2010; King et al. 2012; Li et al. 2013). In Sup35 and Mot3, the prion domain can switch from an intrinsically unfolded state to a prion conformation (i.e., self-templating and infectious fibrils possessing a cross- β -amyloid conformation) (Shorter and Lindquist 2005; Alberti et al. 2009; Halfmann et al. 2012). In yeast, Sup35 and Mot3 prions can be beneficial (Shorter and Lindquist 2005; Halfmann et al. 2012). Thus, by analogy, TDP-43 might also access beneficial prion or amyloid states, which might be involved in RNA-based cellular memories or epigenetic states connected to transcriptional memory (Shorter and Lindquist 2005; King et al. 2012). Here, precedent is provided by another RBP, CPEB, which forms prions via a related prion domain that may underpin long-term potentiation (Si et al. 2003a,b, 2010; Shorter and Lindquist 2005; Majumdar et al. 2012; Drisaldi et al. 2015; Fioriti et al. 2015; Khan et al. 2015; Stephan et al. 2015). Likewise, another RBP with a prion domain, Rim4, forms functional amyloid-like structures that repress translation of cyclin CLB3 in meiosis I, thereby ensuring homologous chromosome segregation (Berchowitz et al. 2015; Ford and Shorter 2015). Regardless, the TDP-43 PrLD is critical for various TDP-43 functions and mediates important protein–protein interactions (Ayala et al. 2005; Buratti et al. 2005; D'Ambrogio et al. 2009; Kim et al. 2010; Kawahara and Mieda-Sato 2012). The PrLD of TDP-43 mediates phase transitions to higher-order liquid- or gel-like multimeric states that likely help organize func-



tional and highly dynamic TDP-43 RNP granules (Johnson et al. 2009; Liu-Yesucevitz et al. 2011; Han et al. 2012; Kato et al. 2012; Wang et al. 2012; Li et al. 2013; Saini and Chauhan 2014; Guo and Shorter 2015; Lin et al. 2015; Molliex et al. 2015). Specifically, the phase transition of the PrLD of TDP-43 is mediated by an α -helix and conserved residues in the 321-340 region (Conicella et al. 2016). Remarkably, the PrLD of TDP-43 can be replaced with the prion domain of Sup35, yielding a TDP-43 hybrid protein that can still assemble into dynamic granules and function in alternative splicing (Wang et al. 2012). The dynamic phase-transitioning behavior mediated by the TDP-43 PrLD enables rapid assembly and disassembly of RNP granules, but is likely to simultaneously render TDP-43 prone to accessing deleterious misfolded states or pathological aggregates that contribute to disease (Hart and Gitler 2012; King et al. 2012; Buchan et al. 2013; Li et al. 2013; Ramaswami et al. 2013; Guo and Shorter 2015; Lin et al. 2015; Molliex et al. 2015).

TDP-43 MISFOLDING AND PATHOGENESIS IN ALS

TDP-43 forms pathological inclusions in degenerating neurons of ALS patients (Arai et al. 2006; Neumann et al. 2006). The inclusions are typically cytoplasmic, and, consequently, TDP-43 is depleted from the nucleus (Arai et al. 2006; Neumann et al. 2006). However, nuclear TDP-43 inclusions can also be observed in neurons and glia (Arai et al. 2006; Neumann et al. 2006). In ALS, TDP-43 shows abnormal phosphorylation, ubiquitylation, aberrant lysine acetylation, and accumulation of C-terminal fragments comprising the PrLD and a portion of RRM2 (Arai et al. 2006; Neumann et al. 2006; Cohen et al. 2015). More than 40 mutations in TDP-43 are now connected to sporadic and familial ALS as well as FTLTDP, and, remarkably, the vast majority of these mutations reside in the PrLD (Gitcho et al. 2008; Kabashi et al. 2008; Sreedharan et al. 2008; Pesiridis et al. 2009; Lattante et al. 2013). The loss of nuclear TDP-43 in degenerating neurons of ALS patients indicates that a loss of nuclear TDP-43 function likely

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contributes to disease (Lee et al. 2012; Li et al. 2013; Ling et al. 2013). Moreover, the sequestration of TDP-43 in misfolded structures in the cytoplasm also indicates a loss of cytoplasmic function as well as a toxic gain of function (Lee et al. 2012; Li et al. 2013; Ling et al. 2013). Indeed, TDP-43 clearance by autophagy induction mitigates neurodegeneration (Barmada et al. 2014; Scotter et al. 2014). In our view, it seems probable that TDP-43 pathogenesis in ALS reflects a combination of loss-of-function and gain-of-toxic function phenotypes (Lee et al. 2012; Li et al. 2013; Ling et al. 2013).

In isolation, pure TDP-43 is intrinsically aggregation prone and upon agitation rapidly assembles into pore-shaped oligomers and fibrils (Johnson et al. 2009; Couthouis et al. 2011; Fang et al. 2014). The pore-shaped TDP-43 oligomers bear remarkable ultrastructural similarity to toxic oligomers formed by other neurodegenerative disease proteins, including α -synuclein and A β (Lashuel et al. 2002). An oligomer-specific antibody, A11, which detects toxic oligomers formed by diverse neurodegenerative disease proteins (Kayed et al. 2003), also recognizes these pure TDP-43 oligomers (Fang et al. 2014). Moreover, purified TDP-43 oligomers are toxic to neurons in culture and also display localized neurotoxicity in vivo when injected directly into the mouse hippocampus (Fang et al. 2014). Similar TDP-43 oligomers can also be detected in the brains of transgenic TDP-43 mice and FTLTDP patients, but not in controls (Fang et al. 2014). These findings indicate that TDP-43 oligomers might contribute to pathogenesis.

It is likely, however, that components of the proteostasis network, including endogenous RNA substrates and interacting proteins, prevent rapid TDP-43 misfolding in vivo. Indeed, RNA ligands, including oligonucleotides from the 3'UTR of the TDP-43 mRNA, prevent TDP-43 aggregation in vitro and preserve TDP-43 in its native dimeric state (Huang et al. 2013; Sun et al. 2014). Accordingly, lysine acetylation events that impair RNA binding or mutation of conserved phenylalanines in the RRM1 (especially RRM1) that engage RNA promote TDP-43 aggregation in cells (Elden et al. 2010;

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Wang et al. 2012; Cohen et al. 2015). However, it is critical to note that simple TDP-43 aggregation alone does not suffice to confer toxicity in multiple model systems (Johnson et al. 2008; Elden et al. 2010; Voigt et al. 2010). Thus, over-expression of RNA-binding defective TDP-43 variants leads to TDP-43 misfolding and aggregation, but not toxicity (Johnson et al. 2008; Elden et al. 2010; Voigt et al. 2010). These findings suggest that RNA binding by TDP-43 and TDP-43 misfolding are required for toxicity (Johnson et al. 2008; Elden et al. 2010; Voigt et al. 2010). Hence, interfering with either RNA binding by TDP-43 or TDP-43 misfolding might be sufficient to alleviate toxicity.

Why is the combination of RNA binding and protein misfolding so critical for TDP-43 toxicity? Misfolded forms of TDP-43 might sequester essential RNA molecules, RBPs, or both to promote neurodegeneration (Polymenidou et al. 2011; Tollervey et al. 2011; Armakola et al. 2012). In fact, misfolding might cause TDP-43 to bind RNA more tightly, as with *Aplysia* CPEB (Si et al. 2003b). RNA could stabilize or divert TDP-43 to access specific misfolded forms that are particularly neurotoxic. Indeed, different RNAs might even engender TDP-43 to fold into different misfolded forms or fibril “strains,” some of which might be extremely toxic. In this regard, it is interesting to note that RNA can enable mammalian PrP to more readily access infectious conformations (Deleault et al. 2003; Wang et al. 2010, 2011a,b). Thus, perhaps certain RNAs enable TDP-43 to populate specific, deleterious self-templating conformers. Further studies at the pure protein level are necessary to test these hypotheses and to define misfolding trajectories followed by TDP-43 under different conditions.

Importantly, the TDP-43 PrLD drives TDP-43 misfolding, as deletion of the PrLD prevents spontaneous TDP-43 aggregation in isolation (Johnson et al. 2009). Indeed, deletion of a short segment (amino acids 311–320) within the PrLD precludes TDP-43 aggregation in vitro (Saini and Chauhan 2011). PrLD deletion prevents aberrant TDP-43 misfolding events and toxicity in various model systems (Johnson et al. 2008; Ash et al. 2010; Wang et al. 2012).

Moreover, increased expression of C-terminal fragments of TDP-43 that harbor the PrLD confers toxicity and cytoplasmic TDP-43 aggregation in several settings (Johnson et al. 2008; Zhang et al. 2009; Ash et al. 2010; Yang et al. 2010; Pesiridis et al. 2011; Caccamo et al. 2012). Interestingly, although not required for misfolding in vitro or in yeast (Johnson et al. 2008, 2009), determinants in the TDP-43 NTD also contribute to the recruitment of full-length TDP-43 to the aggregated state and its subsequent inactivation (Budini et al. 2015; Romano et al. 2015). Moreover, under some conditions in mammalian cells, the N-terminal 10 residues of TDP-43 are important for the cytoplasmic aggregation of TDP-43 variants bearing a mutated NLS that precludes nuclear import (Zhang et al. 2013). These findings raise the possibility that TDP-43 might form a spectrum of distinct misfolded, aggregated structures, which can vary depending on the precise conditions.

Remarkably, nearly all the ALS-linked mutations in TDP-43 are located in the PrLD (Lat-tante et al. 2013). A yeast model of TDP-43 proteinopathy combined with pure protein biochemistry suggests that ALS-linked TDP-43 variants fall into two distinct classes (Johnson et al. 2008, 2009). The first class of ALS-linked TDP-43 mutations has no effect on TDP-43 misfolding in vitro and does not promote toxicity in yeast and includes G294A (Johnson et al. 2009). These findings suggest that some ALS-linked TDP-43 mutations in the PrLD do not impact misfolding events directly. The second class of ALS-linked TDP-43 mutations, including Q331K and M337V, accelerates spontaneous TDP-43 misfolding in isolation and potentiates TDP-43 toxicity in yeast (Johnson et al. 2009). These mutations also alter the phase-separation properties of TDP-43 (Conicella et al. 2016; Schmidt and Rohatgi 2016). Moreover, Q331K shows greater toxicity than WT TDP-43 in *Drosophila* (Elden et al. 2010). Indeed, similar effects of ALS-linked mutations in promoting TDP-43 misfolding and toxicity have been uncovered in diverse model systems, including pure protein settings, cell culture, flies, chicken embryos, mice, and rats (Sreedharan et al. 2008;



Zhang et al. 2009; Barmada et al. 2010; Kabashi et al. 2010; Li et al. 2010; Ritson et al. 2010; Guo et al. 2011; Lim et al. 2016). Collectively, these findings indicate that a subset of ALS-linked TDP-43 variants likely cause disease by a gain-of-toxic function mechanism (Gitler and Shorter 2011).

It is important to note, however, that loss of TDP-43 function also plays a key role in pathogenesis, as selective depletion of TDP-43 from neurons causes neurodegeneration *in vivo* (Feiguin et al. 2009; Wu et al. 2012; Iguchi et al. 2013). Moreover, depletion of TDP-43 from mouse embryonic stem cells causes a defect in repression of cryptic exons and cell death (Ling et al. 2015). Loss of nuclear function is anticipated from the nuclear depletion of TDP-43, as is loss of cytoplasmic function because of TDP-43 sequestration in inclusions (Lee et al. 2012; Li et al. 2013; Ling et al. 2013). However, ALS-linked TDP-43 mutations can also cause loss of cytoplasmic TDP-43 function in the spatially restricted context of functional mRNP granules (Alami et al. 2014; Jovicic and Gitler 2014). TDP-43 assembles into cytoplasmic mRNP granules in neurons that show bidirectional, microtubule-dependent transport, which facilitates delivery of target mRNAs to distal neuronal compartments (Alami et al. 2014; Jovicic and Gitler 2014). The ALS-linked TDP-43 variants A315T, M337V, and G298S impair this transport activity, suggesting a partial loss of this cytoplasmic function (Alami et al. 2014; Jovicic and Gitler 2014). Thus, altered mRNP granule assembly or dynamics might contribute to neurodegeneration, and restoring functional RNP granule assembly, dynamics, or disassembly may have therapeutic utility (Li et al. 2013; Ramaswami et al. 2013).

Does TDP-43 access a bona fide prion or prion-like form? An interesting facet of ALS is the gradual spread of pathology from initiating sites to contiguous areas of the brain, which involves multiple cell types (Ravits and La Spada 2009). This pattern of disease spread has led to suggestions that a prion-like agent might be involved (Cushman et al. 2010; Polymenidou and Cleveland 2011, 2012; Udan and Baloh 2011; King et al. 2012; Grad et al. 2015;

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Maniecka and Polymenidou 2015; Josephs et al. 2016). Intriguingly, phosphorylated TDP-43 pathology in ALS has been interpreted to spread in a sequential manner with highly discernible stages that might indicate involvement of axonal pathways (Brettschneider et al. 2013, 2014; Ludolph and Brettschneider 2015).

However, does the PrLD of TDP-43 enable TDP-43 to access an infectious, self-templating amyloid form just like the prion domains of various yeast prion proteins (Shorter and Lindquist 2005)? Full-length TDP-43 purified under native conditions does not appear to readily form a classic amyloid structure recognized by diagnostic amyloid dyes such as thioflavin T or Congo red (Johnson et al. 2009). Likewise, ALS pathology is typically devoid of amyloid structures recognized by these dyes (Neumann et al. 2007), although, in a subset of ALS cases, TDP-43 skeins are recognized by thioflavin S and contain TDP-43 fibrils (Robinson et al. 2013). Short, synthetic peptides derived from the PrLD of TDP-43 can access toxic amyloid conformers (Chen et al. 2010; Guo et al. 2011). However, these short peptides do not occur naturally, and so their relevance is uncertain. Moreover, the vast majority of proteins harbor short peptides able to form amyloid in isolation (Goldschmidt et al. 2010). Intriguingly, TDP-43 and C-terminal TDP-43 fragments (193–414) purified under denaturing conditions can form fibrils that do not bind thioflavin T, but seed TDP-43 aggregation *in vitro* and in cell culture (Furukawa et al. 2011). Moreover, detergent-insoluble fractions purified from ALS brains containing TDP-43 fibrils induced aggregation of endogenous TDP-43 in neuroblastoma cells in culture (Nonaka et al. 2013). Thus, TDP-43 may indeed be able to access a prion-like conformation, which may even be transmitted across axon terminals (Feiler et al. 2015). However, convincing evidence of transmissible prion-like TDP-43 conformers will require their assembly from purely synthetic protein followed by induction of neurodegenerative disease upon injection into transgenic or WT mice, as has been achieved with PrP and α -synuclein (Legname et al. 2004; Colby et al. 2009; Wang et al. 2010, 2011a,b; Luk et al. 2012).

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AN RBP WITH A PrLD HAS EMERGED IN SEVERAL NEURODEGENERATIVE DISEASE SETTINGS

In addition to TDP-43, another ~70 human RBPs harbor PrLDs of similar low complexity sequence and amino acid composition to domains that drive prionogenesis of yeast proteins like Sup35 (Cushman et al. 2010; King et al. 2012; Kim et al. 2013; March et al. 2016). These include FUS, TAF15, EWSR1, hnRNPA1, and hnRNPA2/B1, which have all surfaced as misfolded RBPs in the etiology of neurodegenerative diseases (Kwiatkowski et al. 2009; Vance et al. 2009; Cushman et al. 2010; Lagier-Tourenne et al. 2010; Couthouis et al. 2011, 2012, 2014; Neumann et al. 2011; Sun et al. 2011; King et al. 2012; Mackenzie and Neumann 2012; Kim et al. 2013; Ling et al. 2013; March et al. 2016). It is now widely thought that PrLDs enable RBP function and mediate phase transitions that partition functional ribonucleoprotein compartments (Kato et al. 2012; Li et al. 2013; Ramaswami et al. 2013). This PrLD activity, however, renders RBPs prone to populating deleterious oligomers or self-templating fibrils that might spread disease, and disease-linked PrLD mutations can exacerbate this risk (Kim et al. 2013; Ramaswami et al. 2013; Shorter and Taylor 2013). One particularly clear case involves mutations in hnRNPA1 and hnRNPA2 that underpin MSP (Kim et al. 2013; Shorter and Taylor 2013). Here, a conserved gatekeeper aspartate residue in the exact same position of the hnRNPA1 or hnRNPA2/B1 PrLD is mutated to valine (Kim et al. 2013; Shorter and Taylor 2013). Thus, the D262V mutation in hnRNPA1 or the D290V mutation in hnRNPA2 introduces a potent amyloidogenic steric zipper into the PrLD that accelerates hnRNP misfolding into self-templating fibrils and cytoplasmic-inclusion formation (Kim et al. 2013; Shorter and Taylor 2013). Remarkably, very similar mutations in the PrLD of hnRNPD, D378N, or D378H have now been linked to limb-girdle muscular dystrophy type 1G (Vieira et al. 2014). We predict that human proteins bearing PrLDs will continue to emerge in the etiology of protein-misfolding disorders and degenerative diseases (King et al. 2012; March et al. 2016).

STABILIZING NATIVE TDP-43 DIMERS AS A THERAPEUTIC STRATEGY

In the closing sections of this review, we consider possible therapeutic approaches to combat TDP-43 proteinopathies. The only small-molecule drug treatment available for neurodegenerative disorders that targets the underlying protein-misfolding events that cause disease is Tafamidis (Bulawa et al. 2012; Nencetti et al. 2013). Tafamidis is used to treat transthyretin (TTR)-related hereditary amyloidosis (also known as familial amyloid polyneuropathy), a rare but deadly neurodegenerative disease (Bulawa et al. 2012; Nencetti et al. 2013). Tafamidis functions by stabilizing the correctly folded, native tetramer of TTR, thereby preventing TTR tetramer dissociation, which is the first and rate-limiting step of TTR amyloidogenesis (Hammarstrom et al. 2003; Hurshman et al. 2004; Bulawa et al. 2012). Based on this strong precedent, a potentially powerful therapeutic strategy might be to isolate small molecules or other agents that stabilize the native dimeric state of TDP-43 and thereby prevent misfolding (Zhang et al. 2013). Interestingly, endogenous RNA ligands may already provide this activity (Sun et al. 2014), which could be mimicked by specific aptamers.

RESTORING NUCLEAR TDP-43 VIA MODULATION OF NUCLEAR TRANSPORT

TDP-43 depletion from the nucleus is widely thought to be a critical event in pathogenesis (Lee et al. 2012; Li et al. 2013; Ling et al. 2013). One potential therapeutic strategy could be to restore nuclear TDP-43 by inhibiting TDP-43 nuclear export or stimulating TDP-43 nuclear import. Thus, small molecules that inhibit expression of nuclear export factors or increase expression of nuclear import factors could be useful. Likewise, small-molecule inhibitors of nuclear export or small-molecule enhancers of nuclear import could be advantageous. Encouragingly, orally administered reversible inhibitors of the nuclear export factor Crm1 (which decodes the TDP-43 NES) signif-

icantly attenuated mouse models of inflammatory demyelination and other models of axonal damage (Haines et al. 2015). Importantly, in neurons, Crm1 inhibitors prevented nuclear export of factors connected with axonal damage while retaining transcription factors in the nucleus that promote neuroprotection (Haines et al. 2015). It will be interesting to test whether these Crm1 inhibitors display efficacy against neuronal or animal models of TDP-43 proteinopathy.

INHIBITING OR REDUCING EXPRESSION OF ATAXIN 2

Genome-wide screens in yeast have uncovered a number of genetic suppressors of TDP-43 toxicity (Elden et al. 2010; Sun et al. 2011; Armakola et al. 2012). Among these, deletion suppressors are of particular interest because they could be targeted by small-molecule inhibitors or siRNA or antisense strategies. Deletion of the yeast homolog of Ataxin 2, Pbp1, suppresses TDP-43 toxicity in yeast (Elden et al. 2010). Moreover, reduced expression of Ataxin 2 in *Drosophila* mitigates TDP-43 toxicity (Elden et al. 2010). Polyglutamine expansions (>34 Qs) in Ataxin 2 cause the neurodegenerative disease spinocerebellar ataxia type 2 (Imbert et al. 1996; Pulst et al. 1996; Lorenzetti et al. 1997). Remarkably, intermediate polyglutamine expansions (27–33 glutamines) in Ataxin 2 are a common genetic risk factor for ALS (Elden et al. 2010; Bonini and Gitler 2011; Lee et al. 2011a,b; Yu et al. 2011; Gispert et al. 2012; Hart and Gitler 2012; Hart et al. 2012). Small-molecule inhibitors of Ataxin 2 could be valuable, but the precise functions of Pbp1 and Ataxin 2 are not completely understood, although Pbp1 interacts with Pab1 and regulates mRNA polyadenylation and stress-granule biogenesis (Mangus et al. 1998). Another strategy would be to reduce Ataxin 2 expression via siRNA or antisense technology. Encouragingly, Ataxin 2 deletion in mice is not lethal and is associated with mild obesity phenotypes (Kiehl et al. 2006; Lastres-Becker et al. 2008), which augurs well for this potential approach.

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INHIBITING OR REDUCING EXPRESSION OF Dbr1

The lariat-debranching enzyme, Dbr1, has also emerged as a deletion suppressor of TDP-43 and FUS toxicity in yeast (Armakola et al. 2012). Dbr1 hydrolyzes 2'–5' prime branched phosphodiester bonds, which occur at the branch point of excised lariat intron RNA, converting them to linear RNA molecules that are then degraded (Chapman and Boeke 1991). When Dbr1 is deleted, intronic lariats accumulate and, surprisingly, co-localize with TDP-43 inclusions in the cytoplasm of yeast (Armakola et al. 2012). Thus, these accumulated intronic lariat RNAs may act as a decoy and engage TDP-43 to prevent the depletion or sequestration of essential cellular RNAs or RBPs (Armakola et al. 2012). Alternatively, lariat RNAs might engage TDP-43 and alter its misfolding pathway such that more benign TDP-43 structures accumulate. Importantly, depletion of Dbr1 from human neuronal cells or primary rat neurons also reduced TDP-43 toxicity (Armakola et al. 2012; Sun and Cleveland 2012). Thus, the effect of reduced Dbr1 levels on TDP-43 toxicity is conserved from yeast to mammals (Armakola et al. 2012). These advances suggest that small molecules that inhibit Dbr1 activity or antisense strategies to reduce Dbr1 expression could have therapeutic utility in TDP-43 and FUS proteinopathies (Figley and Gitler 2013).

TUNING eIF2 α PHOSPHORYLATION TO LIMIT STRESS-GRANULE ASSEMBLY

Stress granules are cytoplasmic RNP compartments where nontranslating mRNAs, as well as factors involved in translation repression and mRNA decay, are partitioned when translation is stalled or impeded in response to environmental stress (Anderson and Kedersha 2008; Li et al. 2013; Ramaswami et al. 2013). TDP-43 co-localizes with stress granules, and it has been proposed that prolonged stress-granule accumulation enables TDP-43 to access pathological conformers that cause neurodegeneration (Li et al. 2013; Ramaswami et al. 2013). Thus, agents that promote stress-granule disassembly

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or inhibit their initial assembly could have therapeutic potential (Li et al. 2013; Ramaswami et al. 2013). Indeed, several genetic modifiers of TDP-43 and FUS toxicity in yeast are involved in stress-granule assembly pathways (Sun et al. 2011; Kim et al. 2014). Interestingly, phosphorylation of the translation initiation factor, eukaryotic translation initiation factor 2 (eIF2 α), decreases protein synthesis and induces stress-granule formation (Thomas et al. 2011). Inhibition of PERK (protein kinase RNA-like endoplasmic reticulum kinase), a critical effector of unfolded protein response, by the small-molecule GSK2606414 reduces eIF2 α phosphorylation (Axten et al. 2012, 2013; Kim et al. 2014). Remarkably, GSK2606414 mitigates TDP-43 toxicity in *Drosophila* and mammalian neurons, presumably by restoring protein synthesis and thereby antagonizing stress-granule formation (Kim et al. 2014). Moreover, orally administered GSK2606414 is profoundly neuroprotective and prevents clinical disease in prion-infected mice, as do other strategies to reduce eIF2 α phosphorylation (Moreno et al. 2012, 2013). Unfortunately, however, GSK2606414 also causes weight loss and mild hyperglycemia, which is likely a result of an inhibition of PERK that is too severe in the pancreas (Harding et al. 2001; Moreno et al. 2013). Thus, alternative strategies to pharmacologically modulate translational inhibition caused by phosphorylated eIF2 α could be opportune. Interestingly, the small molecule *N,N'*-*trans*-(cyclohexane-1,4-diyl)-*bis*-(2-(4-chlorophenoxy)acetamide) (ISRIB) prevents translational inhibition downstream from eIF2 α phosphorylation (Sidrauski et al. 2013). Remarkably, ISRIB conferred neuroprotection in prion-diseased mice without pancreatic toxicity (Halliday et al. 2015). Moreover, ISRIB induces the rapid dissolution of preformed stress granules, thereby releasing sequestered mRNAs for translation (Sidrauski et al. 2015). It will be of great interest to determine whether ISRIB also counters TDP-43 toxicity. Finally, tuning eIF2 α phosphorylation to cytoprotective rather than cytotoxic levels via inhibition of specific regulatory subunits of protein phosphatases (e.g., using guanabenz and Sephin1) has also yielded promising results in restoring protein homeo-

stasis and preventing neurodegeneration (Tsytler et al. 2011; Vaccaro et al. 2013; Das et al. 2015), and should be explored further in TDP-43 proteinopathy models.

ENGINEERED PROTEIN DISAGGREGASES TO REACTIVATE MISFOLDED TDP-43

Another promising therapeutic strategy is to uncover agents that reverse TDP-43 misfolding and restore TDP-43 to native structure and function (Shorter 2008, 2016; Jackrel and Shorter 2015). Such agents would contemporaneously eliminate any deleterious loss of function or toxic gain of function caused by misfolded TDP-43 (Jackrel and Shorter 2015). Moreover, any self-templating TDP-43 conformers that might spread pathology and any intrinsically toxic oligomeric forms of TDP-43 would also be cleared (Jackrel and Shorter 2015). We have engineered potentiated variants of Hsp104, an AAA⁺ ATPase and protein disaggregase from yeast, by introducing single missense mutations into the autoinhibitory middle domain (Jackrel and Shorter 2015). These potentiated Hsp104 variants solubilize preformed TDP-43 fibrils in vitro and suppress toxicity of TDP-43 and ALS-linked variants in yeast (Jackrel and Shorter 2014a,b, 2015; Jackrel et al. 2014a,b; Sweeny et al. 2015; Torrente et al. 2016). Remarkably, potentiated Hsp104 variants also eliminate TDP-43 inclusions and restore nuclear TDP-43 localization in yeast, phenotypes that could be transformative if they were achieved in ALS patients (Jackrel and Shorter 2014a, 2015; Jackrel et al. 2014a). The challenge ahead is to apply engineered Hsp104 variants to neuronal and animal models of TDP-43 proteinopathies (Jackrel and Shorter 2015).

Hsp104 is absent in metazoa (Shorter 2008; Erives and Fassler 2015). Thus, it is also imperative to uncover human protein disaggregases that might also reverse TDP-43 aggregation and restore TDP-43 to the nucleus. Here, two systems are of particular interest. First, Hsp110, Hsp70, Hsp40, and the small heat shock proteins collaborate to dissolve and reactivate proteins trapped in disordered aggregates (Shorter



2011; Duennwald et al. 2012; Rampelt et al. 2012; Mattoo et al. 2013; Torrente and Shorter 2013; Nillegoda et al. 2015). Hsp110, Hsp70, and Hsp40 can also depolymerize preformed amyloid fibrils (Duennwald et al. 2012; Gao et al. 2015). Moreover, Hsp110, Hsp70, and Hsp40 are important for the resolution of stress granules after stress (Cherkasov et al. 2013; Kroschwald et al. 2015; Walters et al. 2015; Jain et al. 2016). Second, the highly conserved AAA⁺ ATPase, VCP, not only can drive clearance of stress granules via autophagy but also can potentially release soluble proteins in the process (Buchan et al. 2013; Ramaswami et al. 2013). Intriguingly, VCP mutations are a cause of ALS (Johnson et al. 2010; Shaw 2010), and ALS-linked VCP variants show defects in stress-granule clearance and perturb TDP-43 proteostasis (Ritson et al. 2010; Buchan et al. 2013; Ramaswami et al. 2013). It will be important to determine whether the Hsp110 system or the VCP system can counter TDP-43 aggregation and toxicity. Moreover, based on earlier studies on Hsp104 (Jackrel and Shorter 2015), it might also be possible to engineer the Hsp110 or VCP system to more effectively reverse TDP-43 misfolding. Finally, it will also be of great interest to uncover small-molecule activators that enhance the disaggregase activity of the Hsp110 system or the stress-granule clearance activity of VCP (Shorter 2016). In this way, we may directly reverse the TDP-43 misfolding events that underpin diverse neurodegenerative disorders.

OUTLOOK

Although there continue to be no effective therapeutics for ALS or other TDP-43 proteinopathies, it is clear that the past several years have yielded a transformative change in our understanding of these diseases and their underlying mechanisms. To develop innovative therapies and uncover novel targets, we need to more fully understand the complexity of TDP-43's structure, function, biology, misfolding, and pathogenesis. This endeavor will require a major focused effort in basic research before translation to the clinic. Moreover, we suggest that thera-

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pies able to combine some of the approaches outlined above could yield synergistic advances similar to combination therapies that have been successfully deployed to restore proteostasis, eliminate yeast prions, and combat cystic fibrosis and HIV (Clavel and Hance 2004; Mu et al. 2008; Roberts et al. 2009; Duennwald and Shorter 2010; Shorter 2010; Wainwright et al. 2015).

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