

# TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration

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**Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are neurodegenerative diseases with clinical and pathological overlap. Landmark discoveries of mutations in the transactive response DNA-binding protein (TDP-43) and fused in sarcoma/translocated in liposarcoma (FUS/TLS) as causative of ALS and FTLD, combined with the abnormal aggregation of these proteins, have initiated a shifting paradigm for the underlying pathogenesis of multiple neurodegenerative diseases. TDP-43 and FUS/TLS are both RNA/DNA-binding proteins with striking structural and functional similarities. Their association with ALS and other neurodegenerative diseases is redirecting research efforts toward understanding the role of RNA processing regulation in neurodegeneration.**

## TDP-43: A COMMON PATHOLOGICAL AND GENETIC DETERMINANT OF AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL LOBAR DEGENERATION

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by the premature loss of upper and lower motor neurons. The progression of ALS is marked by fatal paralysis and respiratory failure with a typical disease course of 1–5 years. Most incidences of ALS are sporadic but ~10% of patients have a familial history. Dominant mutations in the copper/zinc superoxide dismutase 1 (*SOD1*) gene (1) account for ~20% of the familial ALS forms and ~1% of sporadic cases. Other rare familial cases with atypical forms of ALS are due to mutations in multiple genes; however, a large majority of ALS cases do not have a known genetic component (reviewed in 2–4). The identification of *SOD1* mutations initiated the molecular era of ALS research and significant insights into ALS pathogenesis were provided through the identification of pathways directly affected by the toxicity of mutant *SOD1* (reviewed in 2,3,5).

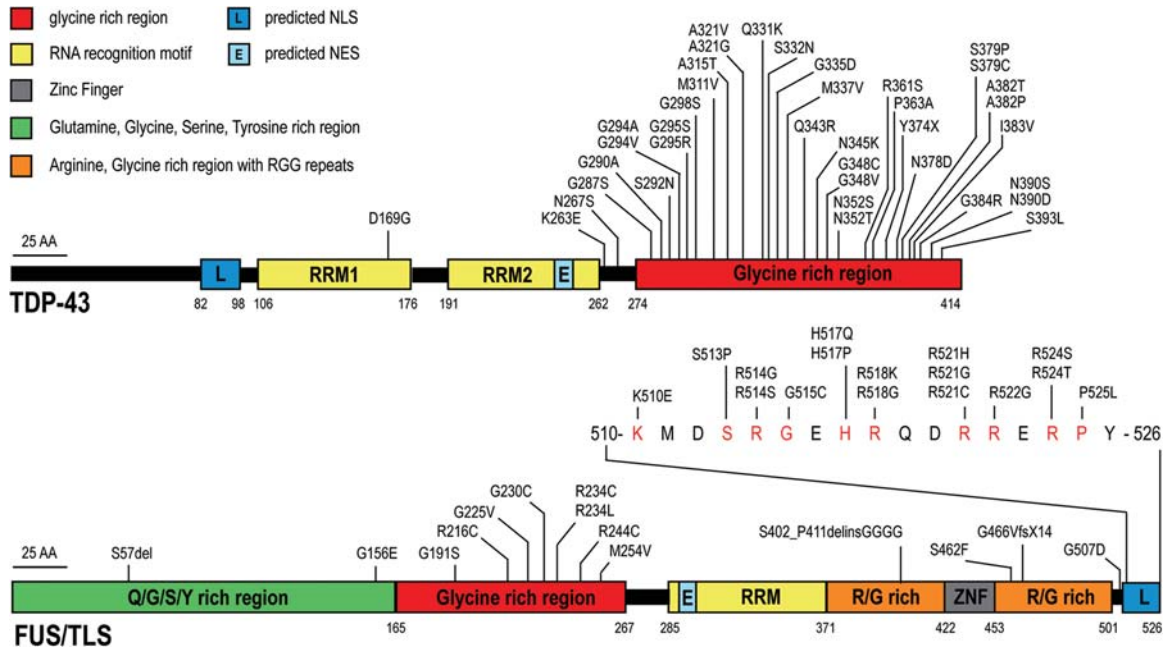
A major shift in our understanding of ALS pathogenesis started in 2006 (6,7) with the identification of the 43 kDa transactive response (TAR) DNA-binding protein (TDP-43) as the main component of ubiquitinated protein aggregates found in

sporadic ALS patients and in patients with frontotemporal lobar degeneration (FTLD), a neurodegenerative disease characterized by behavioral and language disorders (8). Motor neuron disease and cognitive deficits of variable severity can be concomitant in patients or within families (9–12), and the identification of a common pathological hallmark defined by TDP-43- and ubiquitin-positive,  $\alpha$ -synuclein- and tau-negative, cytoplasmic inclusions (6,7) suggested that ALS and FTLD were part of a broad disease continuum, a point now proven by mutations in TDP-43 as causative of either (see below).

Mislocalization of TDP-43 observed by pathology motivated direct sequencing of the gene encoding it (*TARDBP*) in cohorts of patients with motor neuron disease and/or frontotemporal dementia. In early 2008, the successful identification of dominant mutations as a primary cause of ALS provided evidence that aberrant TDP-43 can directly trigger neurodegeneration (13–17). A total of 38 mutations (Fig. 1) have now been described in ALS patients with or without apparent family history, corresponding to ~4% of familial ALS (and <1% of sporadic ALS) (13–33). Most patients with TDP-43 mutation develop a classical ALS phenotype without cognitive deficit, with some variability within families in the site and age of onset. Although initial studies failed to find *TARDBP* mutations in FTLD patients, rare TDP-43 mutations in patients displaying FTLD, with or without motor neuron disease, were reported in 2009 (34–37).

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**Figure 1.** TDP-43 and FUS/TLS mutations in ALS and FTLN patients. Thirty-eight dominant mutations have been identified in TDP-43 in sporadic and familial ALS patients and in rare FTLN patients, with most lying in the C-terminal glycine-rich region. All are missense mutations, except for the truncating mutation TDP-43<sup>Y374X</sup> (upper panel). Thirty mutations have been identified in FUS/TLS in familial and sporadic ALS cases and in rare FTLN patients (R514S and G515S were found in *cis*). Most mutations are clustered in the last 17 amino acids and in the glycine-rich region (lower panel). NLS, nuclear localization signal; NES, nuclear export signal. Domains have been defined according to <http://www.uniprot.org> and <http://www.cbs.dtu.dk/services/NetNES>.

TDP-43 is a 414 amino acid protein encoded by six exons and containing two RNA recognition motifs (RRM1 and 2) and a C-terminal glycine-rich region (38–40). Most of the mutations identified are localized in the glycine-rich region encoded by exon 6 (Fig. 1). All the mutations are dominant missense changes with the exception of a truncating mutation at the extreme C-terminal of the protein (Y374X) (20). Several variants lying in the non-coding regions of the *TARDBP* gene have been identified in patients but further studies are necessary to prove their pathogenic effect (29,35).

Postmortem analysis of patients with TDP-43 mutations found a pattern similar to the TDP-43 pathology described in sporadic ALS and FTLN patients. TDP-43 inclusions are not restricted to motor neurons but can be widespread in brain in ALS patients with or without dementia (16,17,31,41,42). Under normal conditions, TDP-43 is mainly localized within the nucleus, but abnormal TDP-43 distribution such as neuronal cytoplasmic or intranuclear inclusions and dystrophic neurites (6,7), as well as glial cytoplasmic inclusions (7,31,43–45) have been reported. A very curious, and mechanistically unexplained, aspect of TDP-43 pathology is a significant TDP-43 nuclear clearance in a proportion of neurons containing cytoplasmic aggregates, suggesting that pathogenesis may be driven, at least in part, by loss of one or more nuclear TDP-43 functions (6,17,43,46,47). Some ‘pre-inclusions’ have been proposed to arise from diffuse granular cytoplasmic staining and nuclear clearing (17,31,42,43,47–52). Although a plethora of early reports did not establish how prominent this nuclear clearing was and whether it escalates in frequency during disease progression, further efforts, especially the work of

Giordana *et al.* (42), have established that the cytoplasmic redistribution of TDP-43 appears to be an early event in ALS. The pre-inclusions and the larger TDP-43 inclusions are only partially labeled with anti-ubiquitin antibodies (42,52), whereas intranuclear and cytoplasmic inclusions are strongly recognized by phosphorylation-specific antibodies to TDP-43 (33,53–57).

Immunoblotting of detergent-insoluble protein extracts from affected brain and spinal cord has defined a biochemical signature of disease that includes hyperphosphorylation and ubiquitination of TDP-43, and the production of several C-terminal fragments (CTFs) around 25 kDa (6,7). The extent of this pathologic signature correlates with the density of TDP-43 inclusions detected by immunohistochemistry (58,59). Interestingly, the composition of TDP-43 inclusions seems to differ between cortical brain and spinal cord in ALS patients, as inclusions from cortical regions are preferentially labeled by C-terminal antibodies, whereas spinal cord inclusions display equivalent immunoreactivity between N- and C-terminal-specific antibodies (46). In accordance with this, spinal cord extracts showed an absence or only weak accumulation of ~25 kDa CTFs compared with brain extracts (54).

## FUS/TLS: A NEW ACTOR IN ALS AND FTLN

The identification of TDP-43 mutations in ALS was rapidly followed by the discovery of mutations in another RNA/DNA-binding protein, FUS/TLS (fused in sarcoma/translocated in liposarcoma), as a primary cause of familial ALS (60,61).

Thirty mutations (Fig. 1) have now been reported in ~4% of familial ALS and in rare sporadic patients with no apparent familial history (60–74). The inheritance pattern is dominant except for one recessive mutation (H517Q) found in a family of Cape Verdean origin (60). Most are missense mutations with a few exceptions. Indeed, a single amino acid deletion (S57del) (64), a complex 29 bp deletion and 11 bp insertion mutation in exon 12 (p.S402\_P411delinsGGGG) and a *de novo* splice-site mutation leading to the skipping of exon 14 and the production of a truncated FUS/TLS protein (p.G466VfsX14) (74) were identified in sporadic ALS patients. It is noteworthy that in-frame deletions and insertions in polyglycine tracts initially identified in ALS patients (60) were subsequently found in several control individuals, challenging their pathogenic effect (62,64,70). Additionally, further studies are necessary to test whether rare synonymous changes and variants lying in the non-coding regions of *FUS/TLS* gene (63,65,66,71,72) are contributors to pathogenesis.

The site and age of disease onset are variable within families, and incomplete penetrance has been documented for several FUS/TLS mutations (60,63,67,69,72), which may account, at least in part, for the absence of family history in sporadic patients (62,64,66,70,71). Interestingly, several patients harboring the same R521C mutation developed an unusual presentation including an early-onset drop-head syndrome (62,65,67,69). This is an atypical phenotype, as only ~1% of ALS patients present with severe weakness of neck extensor muscles in the early stage of the disease course (75).

Most patients with FUS/TLS mutations develop a classical ALS phenotype without cognitive defect; however, there is one report of a patient who developed FTLN concurrently with motor neuron disease (65) and two patients presented with FTLN in the absence of motor neuron deficit (67,70). At a minimum, these most recent reports provide further evidence that ALS and FTLN have clinical, pathological and genetic overlaps.

FUS/TLS is a 526 amino acid protein encoded by 15 exons (76) and characterized by an N-terminal domain enriched in glutamine, glycine, serine and tyrosine residues (QGSY region), a glycine-rich region, an RRM, multiple arginine/glycine/glycine (RGG) repeats in an arginine- and glycine-rich region and a C-terminal zinc finger motif (Fig. 1) (77–80). Most of the mutations are clustered in the glycine-rich region and in the extreme C-terminal part of the protein with evidence for mutations in each of the five arginine residues present in this region. The FUS/TLS nuclear localization signal (NLS) is likely to reside in this conserved C-terminal region since the NLS of the Ewing sarcoma (EWS) protein, a member of the TET protein family that includes FUS/TLS (79), has been identified in the last 18 amino acid residues which are highly conserved between EWS and FUS/TLS (81). Future studies are necessary to determine if the disease-related mutations clustering in the C-terminal region are responsible for a disruption of the NLS and the abnormal FUS/TLS cytoplasmic redistribution observed in patients.

Like TDP-43, FUS/TLS is mainly nuclear, with lower levels of cytoplasmic accumulation detected in most cell types (82). Postmortem analysis of brain and spinal cord from patients with FUS/TLS mutations found abnormal FUS/TLS

cytoplasmic inclusions in neurons and glial cells (60,61,69). These inclusions were reported to be immunoreactive for FUS/TLS, GRP78/BiP, p62 and ubiquitin, but strikingly not for TDP-43, implying that neurodegenerative processes driven by FUS/TLS mutations are independent of TDP-43 mislocalization (61,69,73). Similar FUS-positive cytoplasmic and intranuclear inclusions were subsequently identified in brain and spinal cord of patients presenting different FTLN subtypes with TDP-43- and tau-negative aggregates (54,83–85). In both ALS and FTLN patients, FUS/TLS nuclear staining was occasionally reduced in neurons bearing cytoplasmic inclusions, but this pattern was less obvious than in TDP-43 proteinopathies and at least some of the normal FUS/TLS nuclear and cytoplasmic staining are retained (61,69,83). Again like TDP-43, ubiquitination of FUS/TLS inclusions has not always been detected, but these inclusions do label strongly with antibodies recognizing the N-terminus, mid-region or C-terminus of the FUS/TLS protein (54,69,83). Immunoblots confirmed the increased levels of full-length FUS/TLS protein in cytoplasmic and insoluble fractions, but no other evidence of biochemical abnormality such as hyperphosphorylation or ubiquitination was found (60,61,83).

## TDP-43 AND FUS/TLS PROTEINOPATHIES: A BROADENING SPECTRUM OF DISEASES

TDP-43 and FUS/TLS mislocalizations have now been observed in a large number of disorders (Table 1), leading to the emergence of a new nomenclature for the set of such diseases: TDP-43 and FUS/TLS proteinopathies (43,86–91). Hallmark TDP-43 misaccumulation is observed in both ALS patients with *TARDBP* mutation and in the vast majority of ALS patients, with the striking exception of familial forms caused by SOD1 mutations and mutant SOD1 transgenic mouse models (45,92–96). TDP-43 proteinopathy is also found in rare cases of FTLN with TDP-43 mutation (36) and in most incidences of sporadic FTLN as well as familial FTLN cases due to mutations in the progranulin (*GRN*) gene, the valosine-containing protein (*VCP*) gene and the unidentified gene on chromosome 9p (6,49,97).

TDP-43 inclusions have also been reported to occur in various forms of dementia, including ~30% of Alzheimer's patients (58,98–103) and various forms of Parkinson's disease (101,103–110). In these instances, TDP-43 inclusions co-exist, but only partially co-localize with, tau or  $\alpha$ -synuclein aggregates (58,98,101–104,111,112). In such 'combined TDP-43 proteinopathies' (57), the mechanism(s) leading to the co-occurrence of aggregations is unknown (58,103). Attempts to correlate the presence or the absence of TDP-43 aggregation with the clinical presentation or severity have produced divergent outcomes (58,103,111–113).

Variability in the extent of TDP-43 pathology among different central nervous system (CNS) regions is observed in the different TDP-43 proteinopathies. The reasons for selective vulnerability are not known nor are TDP-43 misaccumulations restricted to the nervous system. Although TDP-43 localization was reported to be normal in muscle biopsies from ALS patients (114), TDP-43-positive sarcoplasmic inclusions

Table 1. Reported TDP-43 and FUS/TLS inclusions in various diseases

	Disease	TDP-43 inclusions	FUS/TLS inclusions	
<b>Amyotrophic lateral sclerosis</b>	Sporadic ALS	Yes (6,7,42,43,45–47, 52–55,266–271)	No (61,83)	
	With <i>SOD1</i> mutation	No (45,92,106)	No (61,83)	
	With <i>TARDBP</i> mutation	Yes (16,17,31,33,35)	Not tested	
	With <i>FUS/TLS</i> mutation	No (60,61,67,69,73)	Yes (60,61,67,69)	
	With <i>ANG</i> mutation	Yes (272)	Not tested	
<b>Frontotemporal lobar dementia</b>	FTLD-TDP-43	Sporadic or familial with or without MND	Yes (6,7,43,44,46,47,49, 53–55,57,102,151,267,268, 273–278)	No (83,279)
		With <i>GRN</i> mutation	Yes (46,49,53–55,151,280)	No (83,85)
		With <i>TARDBP</i> mutation	Yes (35,36)	Not tested
		With <i>VCP</i> mutation*	Yes (46,49,54,97,115)	No (83,85)
		Linked to chromosome 9p	Yes (49,54,281,282)	No (83)
	FTLD-FUS	Atypical FTLD-U sporadic	No (283–285)	Yes (83,85,279)
		Neuronal intermediate filament inclusion disease	No (49,54)	Yes (83,85)
		Basophilic inclusion body disease	No (49)	Yes (84)
	FTLD-tau	With <i>MAPT</i> mutation	No (47,49,102)	Not tested
		Argyrophilic grain disease	Yes (111)	No (83)
Pick disease		Yes (7,100,267)	No (83)	
Cortico-basal degeneration		Yes (7,58,286)	No (83)	
FTLD-UPS	Progressive supranuclear palsy	No (43,49,58,98)	No (83)	
	With <i>CHMP2B</i> mutation	No (49,287)	No (288)	
<b>Other Dementias</b>	Alzheimer's disease (sporadic and familial)	Yes (7,46,58,98,99,102,112, 113,267,268,289–291)	No (83)	
	Dementia with Lewy's bodies with or without Alzheimer's disease	Yes (102,103,112,267)	No (122)	
	Down syndrome	Yes (290)	Not tested	
	Hippocampal sclerosis dementia	Yes (49,87,98)	Not tested	
	Familial British dementia	Yes (292)	Not tested	
	Prion diseases	No (293)	Not tested	
<b>Parkinsonism</b>	Parkinson's disease with and without dementia	Yes (103,267)	No (83)	
	Parkinson's disease with <i>LRKK2</i> mutation	Yes (107)	Not tested	
	Perry syndrome with <i>DCTN1</i> mutation	Yes (108–110)	Not tested	
	ALS Parkinsonism–dementia complex of Guam	Yes (101,104–106)	Not tested	
<b>Polyglutamine diseases</b>	Huntington's disease	Yes (123)	Yes (83,85,121)**	
	SCA1	Not tested	Yes (85,122)**	
	SCA2	Not tested	Yes (122)**	
	SCA3	Yes (124)	Yes (85,122)**	
	DRPLA	Not tested	Yes (122)**	
<b>Myopathies</b>	Sporadic inclusion body myositis	Yes (115–118)***	Not tested	
	Inclusion body myopathy with <i>VCP</i> mutation*	Yes (115,117,118)***	Not tested	
	Oculo-pharyngeal muscular dystrophy with <i>PABP2</i> mutation	Yes (116)***	No (85)	
	Distal myopathy with rimmed vacuoles	Yes (116)***	Not tested	
	Myofibrillar myopathies with <i>MYOT</i> or <i>DES</i> mutation	Yes (117,118)***	Not tested	
<b>Other diseases</b>	Multiple system atrophy (MSA)	No (7,49,98)	No (83,85)	
	FXTAS	Not tested	No (85)	
	Narcolepsy-cataplexy	No (294)	Not tested	
	Schizophrenia	No (295)	Not tested	

Inclusions of TDP-43 and FUS/TLS have been identified in a growing list of pathological conditions. In most instances, only a subset of patients present with aggregations of TDP-43 or FUS/TLS (e.g. around 30% of Alzheimer's disease patients), leading to inconsistency between different reports. Yes, some patients with the corresponding disease were reported to present with aggregations; No, none of the patients tested displayed abnormal inclusions of TDP-43 or FUS/TLS. Yellow, TDP-43 inclusions; Blue, FUS/TLS inclusions; Green, both TDP-43 and FUS/TLS inclusions; Gray, not tested; MND, motor neuron disease; SCA, spinocerebellar ataxia; DRPLA, dentatorubral-pallidolusian atrophy; FXTAS, fragile X tremor/ataxia syndrome.

\*FTD with inclusion body myopathy and Paget's disease.

\*\*Mainly neuronal intranuclear inclusions (NII).

\*\*\*Sarcoplasmic inclusions.

have been described in various forms of myopathies with inclusion bodies and rimmed vacuoles including those due to *VCP* mutation (115–118). As we go to press, a very provocative doubling (to nearly 100%) of the proportion of all cells in the skin that accumulate TDP-43 has been reported in 15 examples of sporadic ALS (119).

The extent of FUS/TLS proteinopathies is less well defined. However, soon after mutant FUS/TLS was found to aggregate in ALS and FTLN patients, inclusions containing wild-type FUS/TLS protein were reported in three different forms of sporadic FTLN, namely atypical FTLN-U (83), neuronal intermediate filament inclusion disease (120) and basophilic inclusion body disease (84). Moreover, an unbiased proteomic approach identified FUS/TLS as a major component of the intranuclear polyQ aggregates in cellular models for the Huntington and spinocerebellar ataxia type 3 (SCA3) diseases (121). Postmortem analysis of patients with different polyQ diseases (Huntington's disease, SCA1, SCA2 and SCA3 and dentatorubral-pallidolusian atrophy) confirmed the association of FUS/TLS mainly with neuronal intranuclear inclusions and in rare occasions with small cytoplasmic inclusions (83,85,121,122). This led to the provocative proposal that FUS/TLS directly binds to polyQ aggregates in an early stage of amyloid formation (121).

In the vast majority of cases, the occurrence of TDP-43- or FUS/TLS-positive inclusions appears to be mutually exclusive. One exception may be the polyglutamine diseases, since besides FUS/TLS-positive intranuclear inclusions, two groups have reported the presence of TDP-43-positive cytoplasmic inclusions in Huntington's and SCA3 patients (123,124). Further studies using phospho-specific antibodies to TDP-43 are needed to establish if co-occurrence of TDP-43 and FUS/TLS misaccumulation is a consistent feature of these diseases.

## TDP-43 AND FUS/TLS ARE INVOLVED IN MULTIPLE STEPS OF RNA PROCESSING

The precise roles of TDP-43 and FUS/TLS are not fully elucidated. Although there is no evidence that TDP-43 and FUS/TLS act together, they are both structurally close to the family of heterogeneous ribonucleoproteins (hnRNPs) and have been involved in multiple levels of RNA processing including transcription, splicing, transport and translation (Fig. 2A). Such multifunctional proteins could have roles in coupling transcription with splicing and other RNA processes (82,125–129).

### Structure and nucleic acid properties of TDP-43 and FUS/TLS

TDP-43 contains two RRM (RRM1 and 2) and a C-terminal glycine-rich region (38–40). The RRM1 of TDP-43 is indispensable for binding to single-stranded RNA with a minimum of five UG repeats (40,130), and its affinity increases with repeat length (40). Competition assays with synthetic oligonucleotides have suggested that TDP-43 also binds single-stranded, but not double-stranded, DNA with TG repeats (131,132). However, TDP-43 can bind to both single- and double-stranded TG-repeat DNA in nitrocellulose

filter-binding assays, albeit with slightly higher affinity for single-stranded DNA (133).

The function of RRM2 is still obscure, since it is dispensable for RNA binding (130), but has been proposed to play a role in chromatin organization (134). RRM2 may mediate interaction with DNA since this region can co-crystallize with a TG-rich single-stranded DNA so as to form highly thermal-stable dimeric assemblies (133). A proportion of TDP-43 in cells forms a dimer (135), suggesting a possible functional relevance of the RRM2-mediated dimerization of TDP-43.

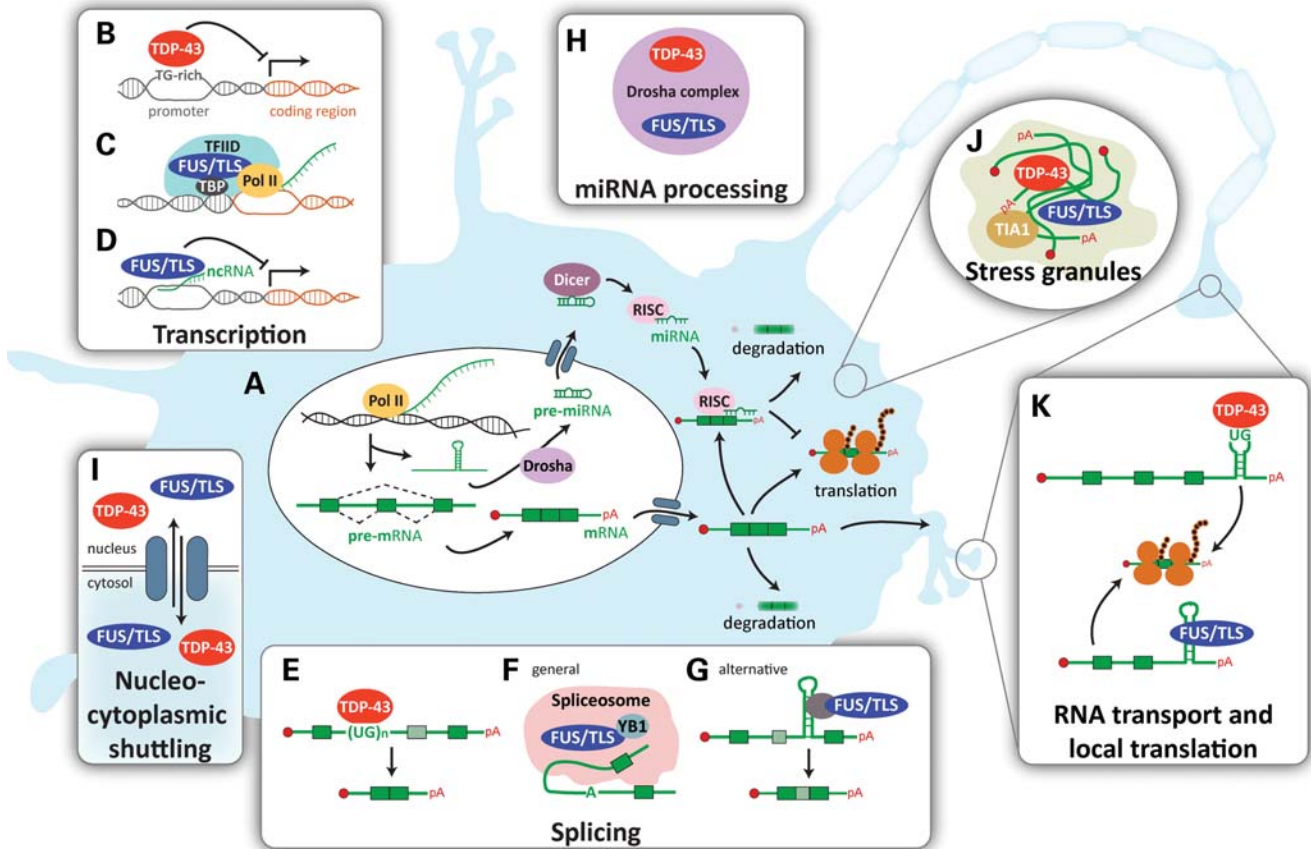
The C-terminal glycine-rich region of TDP-43 is essential for interactions with other proteins (39,40,136). In the native protein state, this domain lacks secondary or tertiary structure, suggesting that it might undergo structural transitions upon binding to its protein partners *in vivo* (137).

FUS/TLS is a member of the TET protein family that also includes the EWS protein, the TATA-binding protein (TBP)-associated factor (TAF<sub>II</sub>68/TAF15) and the *Drosophila* cabeza/SARF protein (79,125). FUS/TLS, EWS and TAF<sub>II</sub>68/TAF15 have a similar structure characterized by an N-terminal QGSY-rich region, a highly conserved RRM, multiple RGG repeats, which are extensively dimethylated at arginine residues (138) and a C-terminal zinc finger motif (Fig. 1) (77–80). FUS/TLS was initially identified as a fusion protein caused by chromosomal translocations in human cancers (77,139). In these instances, the promoter and N-terminal part of FUS/TLS is translocated to the C-terminal domain of various DNA-binding transcription factors conferring a strong transcriptional activation domain to the fusion proteins (125,140).

FUS/TLS was independently identified as the hnRNP P2 protein, a subunit of a complex involved in maturation of pre-mRNA (141). Consistently, *in vitro* studies have shown that FUS/TLS binds RNA, single-stranded DNA and (with lower affinity) double-stranded DNA (77,78,125,142–145). The sequence specificity of FUS/TLS binding to RNA or DNA has not been well established (129); however, using *in vitro* selection (SELEX), a common GGUG motif has been identified in approximately half of the RNA sequences bound by FUS/TLS (146). A later proposal was that the GGUG motif is recognized by the zinc finger domain and not the RRM (80). Additionally, FUS/TLS has been found to bind a relatively long region in the 3' untranslated region (UTR) of the actin-stabilizing protein Nd1-L mRNA, suggesting that rather than recognizing specific short sequences, FUS/TLS interacts with multiple RNA-binding motifs or recognizes secondary conformations (147). FUS/TLS has also been proposed to bind human telomeric RNA (UUAGGG)<sub>4</sub> and single-stranded human telomeric DNA *in vitro* (148,149).

### Role of TDP-43 and FUS/TLS in transcription regulation

TDP-43 was originally identified as a transcriptional repressor that binds to TAR DNA of the human immunodeficiency virus type 1 (HIV-1) (38), hence its name. TDP-43 was also found to bind the promoter of the mouse SP-10 gene that is required for spermatogenesis (132,150). In both instances, TDP-43 represses transcription by binding these DNA regulatory elements (Fig. 2B)—TAR DNA sequence or mouse SP-10 promoter—but little is known about the mechanisms of this



**Figure 2.** Proposed physiological roles of TDP-43 and FUS/TLS. (A) Summary of major steps in RNA processing from transcription to translation or degradation. (B) TDP-43 binds single-stranded TG-rich elements in promoter regions thereby blocking transcription of the downstream gene [shown for TAR DNA of HIV (38) and mouse SP-10 gene (132,150)]. (C) FUS/TLS associates with TBP within the TFIID complex suggesting that it participates in the general transcriptional machinery (156–159). (D) In response to DNA damage, FUS/TLS is recruited in the promoter region of cyclin D1 (*CCND1*) by sense and antisense non-coding RNAs (ncRNAs) and represses *CCND1* transcription (145). (E) TDP-43 binds a UG track in intronic regions preceding alternatively spliced exons and enhances their exclusion [shown for CFTR (38,40,96,130,131,160) and apolipoprotein A-II (161)]. (F) FUS/TLS was identified as a part of the spliceosome (168–170) and (G) was shown to promote exon inclusion in H-ras mRNA, through indirect binding to structural regulatory elements located on the downstream intron (177). (H) Both proteins were found in a complex with Drosha, suggesting that they may be involved in miRNA processing (183). (I) Both TDP-43 and FUS/TLS shuttle between the nucleus and the cytosol (134,142) and (J) are incorporated in SGs where they form complexes with mRNAs and other RNA binding proteins (82,166,190,201–204). (K) TDP-43 and FUS/TLS are both involved in the transport of mRNAs to dendritic spines and/or the axonal terminal where they may facilitate local translation (187–189,191). Examples of such cargo transcripts are the low molecular weight NFL for TDP-43 (51,208) and the actin-stabilizing protein Nd1-L for FUS/TLS (147).

transcriptional repression (reviewed in 96). Consistent with its role in transcription, TDP-43 was found in human brain (151) and in cell culture systems (134,152) to associate with euchromatin—containing actively transcribed genes—and its RRM2 was proposed to mediate this localization (134).

FUS/TLS was found to associate with both general and more specialized factors to influence the initiation of transcription (128). Indeed, FUS/TLS interacts with several nuclear hormone receptors (153) and with gene-specific transcription factors such as Spi-1/PU.1 (154) or NF- $\kappa$ B (155). It also associates with the general transcriptional machinery and may influence transcription initiation and promoter selection by interacting with RNA polymerase II and the TFIID complex (Fig. 2C) (156–158). Recently, FUS/TLS was also shown to repress the transcription of RNAP III genes and to co-immunoprecipitate with TBP and the TFIIB complex (159).

In addition to its direct interaction with the transcriptional machinery, a recent study provided a direct link between FUS/TLS RNA-binding properties and its role in transcription regulation. Indeed, in response to DNA damage, FUS/TLS is recruited by sense and antisense non-coding RNAs transcribed in the 5' regulatory region of the cyclin D1 (*CCND1*) gene. An RNA-dependent allosteric modification of FUS/TLS results in inhibition of CREB-binding protein and p300 histone acetyltransferase activities leading to the repression of cyclin D1 transcription (Fig. 2D) (145).

#### Role of TDP-43 and FUS/TLS in splicing regulation

Evidence for a role of TDP-43 and FUS/TLS in splicing regulation came from the identification of their association with other splicing factors and that their depletion or overexpression affects the splicing pattern of specific targets. One of

the best-characterized examples of alternative splicing regulation for TDP-43 is that of the cystic fibrosis transmembrane regulator (CFTR). The pre-mRNA of CFTR contains an intronic UG tract that is recognized by TDP-43, thereby promoting skipping of exon 9 in CFTR mRNA (Fig. 2E) (39,40,96,130,131,160). TDP-43 was also shown to affect the splicing of apolipoprotein A-II and survival motor neuron (SMN) transcripts (161,162). Alternative splicing coupled with the nonsense-mediated decay (NMD) machinery can sometimes regulate expression levels as in the case of SC35, a serine-/arginine-rich spliceosomal protein. Increased protein levels of SC35 activate splicing events of its own pre-mRNA, resulting in isoforms with premature stop codons that are degraded by the NMD system (163). These splicing events are activated via multiple low-affinity SC35-binding sites and are inhibited by TDP-43 that competes for binding to the same regulatory sequence as SC35 (164).

The association of TDP-43 with a number of proteins involved in splicing (165,166), including SC35 (167), is consistent with its function as a splicing regulator. These interactions are mediated by the C-terminal glycine-rich domain of TDP-43 and truncated TDP-43 proteins that lack this domain do not possess CFTR exon-skipping activity (39,40). Moreover, a TDP-43 interactor protein, hnRNP A2, is a crucial component of splicing regulation, since its knockdown in cells inhibits the exclusion of CFTR exon 9 (136).

Less is known about the role of FUS/TLS in splicing regulation. Proteomic analysis identified this protein as part of the spliceosome machinery (Fig. 2F) (168–170). FUS/TLS associates *in vitro* with large transcription–splicing complexes that bind the 5' splice sites of pre-mRNA (127) and have been also proposed to directly bind pre-mRNA 3' splice site (171). Furthermore, FUS/TLS associates with other splicing factors, including YB-1 (172,173), serine–arginine proteins (SC35 and TASR) (158,174,175), polypyrimidine tract-binding protein (175) or hnRNP A1 and C1/C2 (140). Finally, FUS/TLS overexpression alters the splicing of co-transfected reporter plasmids (146,154,158,172,173,176), and it has recently been proposed to influence alternative splicing of the H-ras mRNA (Fig. 2G) (177).

Despite evidence that TDP-43 and FUS/TLS are involved in RNA splicing (96,128), few of their respective RNA targets have been identified and a comprehensive protein–RNA interaction map still needs to be defined. Recent technologies coupled with high-throughput sequencing have yielded a global insight into RNA regulation (178,179), and such approaches are eagerly anticipated to understand the role of TDP-43 and FUS/TLS in neurodegeneration. Indeed, disrupting the function of an RNA-binding protein can affect many alternatively spliced transcripts, and a growing number of neurological diseases have been linked to this process. Importantly, splicing alterations (180,181) and mRNA-editing errors (182) have been reported in sporadic ALS patients, albeit a role of TDP-43 or FUS/TLS in these modifications has not been explored. The observation of a widespread mRNA splicing defect in TDP-43 and/or FUS/TLS proteinopathies would reinforce the crucial role of splicing regulation for neuronal integrity and potentially identify candidate genes whose altered splicing is central to ALS pathogenesis.

### TDP-43 and FUS/TLS are involved in micro-RNA processing

Both TDP-43 and FUS/TLS may play roles in micro-RNA (miRNA) processing. Both have been found (by mass spectrometry) to associate with Drosha (Fig. 2H) (183), the nuclear RNase III-type protein that mediates the first step in miRNA maturation (reviewed in 184). In addition, TDP-43 may be involved in the cytoplasmic cleavage step of miRNA biogenesis—mediated by the Dicer complex—as suggested by its association with proteins known to participate in these functions, such as argonaute 2 and DDX17 (166). Much more can be anticipated soon on the possible involvement of TDP-43 and FUS/TLS in miRNA processing.

### Cytosolic roles of TDP-43 and FUS/TLS in regulation of RNA subcellular localization, translation and decay

TDP-43 and FUS/TLS are also present in the cytosol where they are involved in diverse aspects of RNA metabolism, regulating the spatiotemporal fate of mRNA, i.e. subcellular localization, translation or degradation. Indeed, both proteins have been shown by interspecies heterokaryon assays to shuttle between the nucleus and the cytoplasm (Fig. 2I) (134,142). In particular, FUS/TLS was shown to relocalize to the cytoplasm upon inhibition of RNA polymerase II transcription (140,185) and post-translational modifications such as tyrosine phosphorylation by the fibroblast growth factor receptor-1 (FGFR1) kinase (186) or dimethylation of arginine residues may alter its subcellular localization (129,138).

In neurons, TDP-43 and FUS/TLS are found in RNA-transporting granules translocating to dendritic spines upon different neuronal stimuli (187–191). In addition, the loss of TDP-43 reduces dendritic branching as well as synaptic formation in *Drosophila* neurons (192,193), and cultured hippocampal neurons from FUS/TLS knockout mice (194) displayed abnormal spine morphology and density (190). Collectively, these results suggest that both proteins could play a role in the modulation of neuronal plasticity by altering mRNA transport and local translation in neurons. In particular, FUS/TLS was found to associate with *N*-methyl-D-aspartate receptor–adhesion protein signaling complexes (189,195,196), suggesting that it participates in the regulation of mRNA translation at excitatory synaptic sites. The accumulation of FUS/TLS in spines is facilitated by myosin-Va (197) and myosin-VI (198) and is dependent on the post-synaptic activation of signaling pathways initiated by the metabotropic glutamate receptor mGluR5 (190). FUS/TLS was also found to bind mRNAs encoding actin-related proteins, such as actin-stabilizing protein Nd1-L, and may be involved in actin reorganization in spines (Fig. 2K) (147). FUS/TLS involvement in the regulation of localized protein synthesis was also suggested by its accumulation with other RNA-binding and ribosomal proteins in the spreading initiation centers of adhering cells (82,199). The role of TDP-43 in the regulation of local translation is less well established; however, it was shown to act as a translational repressor *in vitro* (200) and has extensive interactions with proteins participating in translation (166).

Increasing evidence suggests that TDP-43 and FUS/TLS are integral components of RNA stress granules (SGs; Fig. 2J) (82,166,200–204), cytoplasmic, microscopically visible foci consisting of mRNA and RNP complexes that stall translation under stress conditions (reviewed in 205). However, in contrast to TIA1 and TIAR, two bona fide components of SGs (206), TDP-43 is neither essential for formation of SGs nor a neuroprotective factor in stress conditions (202). Nonetheless, in axotomized motor neurons *in vivo*, TDP-43 was found to translocate to the cytoplasm where it formed SGs that dissolved after neuronal recovery (203,207). The role of these TDP-43-positive SGs remains unknown, but they were proposed to mediate the stabilization and transport of the low molecular weight neurofilament (NFL) mRNA to the injury site for local translation of NFL protein required for axonal repair (51,208).

### ROLES OF TDP-43 AND FUS/TLS IN DEVELOPMENT AND/OR MAINTENANCE OF GENOME STABILITY

TDP-43 is essential for early mouse embryogenesis as mice with homozygous disruption in the *Tardbp* gene are embryonically lethal (209–211), a consequence of defective outgrowth of the inner cell mass prior to implantation (210). TDP-43 is developmentally regulated, since the levels of TDP-43 are sustained throughout embryonic development, but in post-natal brains, its accumulation gradually decreases with age (210). TDP-43 expression seems to be tightly regulated, since the heterozygous *Tardbp* gene disruption mice have apparently unchanged protein levels and are developmentally indistinguishable from control littermates (209–211). In agreement with the above *in vivo* results, knockdown of TDP-43 induces cell death in cultured tumor cells and differentiated neurons (212,213). TDP-43 disruption in *Drosophila* leads to lethality at second larvae stage (214), although other groups reported ‘semi-lethal’ phenotypes, with some TDP-43 null flies surviving through adulthood, suggesting additional compensatory mechanisms that might be specific to the fly (192,193).

Two independent sets of mice with disruption of the *Fus/Tls* gene have been produced by the insertion of gene-trap constructs in exon 8 or 12, respectively (194,215). Recognizing that low levels of a truncated protein are produced, *Fus/Tls*<sup>+/-</sup> heterozygous mice do not display overt abnormality, whereas the phenotype of the *Fus/Tls*<sup>-/-</sup> gene disruption mice varies depending on the genetic background. Inbred *Fus/Tls*<sup>-/-</sup> mice have been reported to be small at birth and die within a few hours with major defects in B-lymphocyte development (194), whereas similar mice in an outbred background survive until adulthood and develop male sterility (215). Murine embryonic fibroblasts derived from the *Fus/Tls*<sup>-/-</sup> gene disruption mice display high chromosomal instability and radiation sensitivity (194,215). In fact, several lines of evidence suggest that FUS/TLS is required for the maintenance of genomic integrity and may have a role in DNA double-strand break repair. Indeed, FUS/TLS promotes the annealing of homologous DNA and the formation of DNA D-loops, an essential step in DNA repair by homologous recombination (144,216,217).

FUS/TLS is a key transcriptional regulatory sensor of DNA-damage signals, as exemplified by its recruitment to the cyclin D1 promoter after ionizing radiation exposure where it functions as a transcriptional repressor (145). Moreover, FUS/TLS is phosphorylated by ATM (ataxia-telangiectasia mutated) upon induction of double-strand breaks (218) and mouse models with disruption of FUS/TLS present high levels of chromosomal instability, male sterility due to defects in the formation of autosomal bivalents and increased sensitivity to ionizing irradiation (194,215).

### UNDERSTANDING THE PATHOLOGICAL MECHANISMS OF DISEASE: CELLULAR AND ANIMAL MODELS FOR TDP-43

The role of TDP-43, FUS/TLS and mutations thereof in the pathogenesis of the respective proteinopathies has not been established and both a gain of toxic property(ies) and a loss of function via their sequestration in aggregates are plausible. Animal models for the most recently identified FUS/TLS have not yet been reported, whereas for TDP-43, the first outcomes modeling disease in fruit flies or mice have produced a confusing story that has not yet settled the key questions concerning mutant TDP-43-mediated pathogenesis.

#### Wild-type and mutant TDP-43 in disease

Expression of wild-type human TDP-43 has been found to cause neurodegeneration in *Drosophila* (192,219,220) and rodent models (221,222). In *Drosophila*, human TDP-43-mediated neurodegeneration was associated with impaired locomotive activity (219), paralysis and reduced life span (220), whereas in mice it presented as a very rapidly progressive paralysis reminiscent of human ALS in a dose-dependent manner (222). In addition, overexpression of wild-type TDP-43 in primary neurons led to cell death independently of mutation (223).

Although the above observations argue for a pathogenic role of elevated wild-type TDP-43 levels, it remains unclear whether increased levels of TDP-43 are a common finding in human patients with TDP-43 proteinopathies. So far, no copy number variation of the *TARDBP* gene has been found (19,25,224,225). Brain mRNA levels of TDP-43 were also found unchanged in a handful of patients with TDP-43 proteinopathies, with the exception of a single patient with a 3' UTR variant of TDP-43 that showed a 2-fold increase in TDP-43 expression levels compared to controls (35). It is noteworthy that elevated levels of TDP-43 mRNA are found in the Wobbler mouse that develops motor neuron disease (226).

Expression of human TDP-43 carrying disease-associated mutations was suggested to exhibit higher toxicity than the wild-type protein in chick embryos (14) and primary rodent neurons (223,227). Mutant TDP-43, but not its wild-type counterpart, caused a motor phenotype in zebrafish associated with shorter and disorganized axons with excessive branching (227). Recently, accumulation to ~3-fold of the endogenous level of mutant TDP-43 carrying the A315T mutation in mice was reported to trigger a phenotype including gait abnormalities



consistent with upper motor neuron disease (228). Since these observations were made in a single transgenic line, it is impossible to exclude integration site artifacts or to determine the role of expression levels or the presence of the point mutation in the induction of the phenotype in this study. These are all crucial points that await independent validation.

Despite the usage of neuronal or more broadly expressed prion protein promoters to drive the expression of transgenes, both wild-type (222) and mutant (228) transgenes have produced loss of cortical layer V pyramidal neurons in the frontal cortex, suggesting a selective vulnerability of this set of neurons to dose-dependent, human TDP-43-mediated toxicity. Moreover, this cortical area exhibited extensive microgliosis and astrocytosis in both mutant and wild-type mouse models (222,228).

Most recently a novel rat model with overexpression of mutant—but not wild-type—human TDP-43 was shown to develop widespread neurodegeneration primarily in the motor system leading to progressive paralysis (229).

### Cytoplasmic localization of TDP-43 and intracellular aggregates

Increased cytoplasmic localization of TDP-43 in brains and spinal cords of patients—designated ‘pre-inclusions’—was proposed to be an early event in TDP-43 proteinopathies (42), with the implication of a possible pathogenic role. Consistently, increased cytoplasmic TDP-43 localization was found at presymptomatic stages in mice overexpressing wild-type TDP-43 (222) as well as in an acute rat model with adenovirus-mediated wild-type TDP-43 expression (221). In various cell culture experiments, the expression of TDP-43 proteins carrying mutations that disrupt its NLS (amino acids 78–84) led to localization primarily within the cytoplasm (223,230,231). An elegant study utilized an automated microscopy system for long-term visualization and quantitative correlation between morphologic changes and survival of individual neurons to show that cytoplasmic TDP-43 is toxic for rat primary cortical neurons (223). Although overexpression of wild-type TDP-43 led to increased cytoplasmic localization of TDP-43 and cell death independently of mutation, pathogenic TDP-43 mutations increased the proportion of cytoplasmic TDP-43 (223).

On the other hand, how forced synthesis of high levels of TDP-43 relates to actual pathogenic mechanism for the lower levels of TDP-43 in the physiologically relevant contexts is not established by such approaches. TDP-43 is likely to perform one or more cytoplasmic roles including contribution to neuronal recovery as illustrated by the rapid, transient cytoplasmic translocation of TDP-43 in response to stress (203,207). These observations led to the hypothesis that prominent cytosolic localization in neurons of ALS or FTL-D-U patients may actually represent the typical response to stress rather than an initiating event in pathogenesis (203,207). This could provide a partial explanation for the remarkably common incidence of cytoplasmic TDP-43 accumulation in a variety of neurodegenerative conditions of seemingly different origins (Table 1).

Increased cytoplasmic localization of TDP-43 is associated with the formation of intracellular aggregates in the affected areas in patients (6,7,42) and in animal models including rats

(221), mice (222) and *Drosophila* (219). In cell culture systems, increased cytoplasmic localization of TDP-43 was proposed to facilitate the formation of intracellular aggregates (223,230,231), albeit this argument suffers from a ‘chicken versus the egg’ ambiguity. So too do arguments concerning the role of the inclusions in pathogenesis. As in most neurodegenerative diseases where cytoplasmic aggregations are evident, an unresolved controversy is whether inclusions are neurotoxic or neuroprotective (232), the latter presumably through the sequestration of smaller toxic species of misfolded proteins. For example, early studies of hTDP-43 mutants (233,234) in yeast reported that foci of aggregated protein were better determinants of cellular toxicity than diffuse cytosolic staining, but this was not seen for inclusion body formation in the cytoplasm of primary neurons (223) or in *Drosophila* motor neurons *in vivo* (220). In any event, the possibility that neurotoxicity of aggregates might stem from the sequestration of essential proteins within the inclusions does not include some known binding partners of TDP-43 (e.g. hnRNP A1, A2/B1 and C and SMN), as these appear to be absent from the inclusions (235).

### Phosphorylation and C-terminal fragmentation of TDP-43

The role of phosphorylation of TDP-43 in FTL-D-U and ALS patients has been explored with the help of phospho-specific antibodies that strongly bind to nuclear and cytoplasmic TDP-43 inclusions without recognizing normal intranuclear TDP-43 (53–56). Using these, S409/410 have been identified as the major sites of phosphorylation on TDP-43 (55) and were found highly phosphorylated in the proposed pathologic 25 kDa CTFs (see below) (53–56). It is unclear whether phosphorylation of TDP-43 and its CTFs are a primary event, or an epiphenomenon following earlier pathogenic events. Although a correlation between insolubility and phosphorylation of TDP-43 has been reported (236), phosphorylation is not required for C-terminal cleavage, aggregation or toxicity, at least in cellular models (237,238).

Fragments (20–25 kDa) containing the carboxy-proximal portion of TDP-43 accumulate in detergent (sarkosyl)-insoluble fractions derived from patient CNS tissues (6,7). These CTFs originate—at least in part—from proteolytic cleavage at Arg208 (236) and are more prominent in brains of FTL-D-U and ALS patients, whereas in the spinal cord of both groups, the predominant species within the inclusions are full-length TDP-43 (46). When expressed in cells, the 25 kDa CTFs recapitulate some of the pathological features of TDP-43 proteinopathy such as increased cytoplasmic accumulation, insolubility, hyperphosphorylation, polyubiquitination and cytotoxicity (46,237,239). In transgenic mice expressing wild-type or mutant TDP-43, the appearance of the 25 kDa CTF (222,228) was shown to increase with disease progression, arguing for a pathogenic role (222). Curiously, in contrast to observations in patients (6,7,46) and in cell culture (204,237,238,240), where 25 kDa CTF is mainly cytoplasmic, the same fragment in transgenic mice was present solely in the nucleus where it formed intranuclear inclusions (222).

Caspase-3, which is activated during apoptosis, has been proposed to be the main protease generating the 25 kDa CTF in cells (204,237,238,240), albeit if so then its production would seem to be a late event as caspase-3 is widely con-

sidered to be an executioner caspase whose activation initiates imminent cell death. Recognizing this, a further proposal has emerged that partial caspase-3 activation and subsequent proteolytic processing of TDP-43 occur not only upon induction of apoptosis, but also when progranulin, a secreted growth factor, is downregulated (204,237,240), albeit this was not replicated by others (203,238,241). Despite the above discrepancy, there are independent clues that support a link between progranulin downregulation and TDP-43 cytoplasmic accumulation and possibly also TDP-43 fragmentation. In familial FTL-D-U patients with progranulin mutations, all of which lead to progranulin loss of function or haploinsufficiency (242–244), TDP-43 aggregation and C-terminal fragmentation are evident (49). Moreover, TDP-43 cytoplasmic accumulations were reported in progranulin knockout mice (245), albeit the proteolytic processing of TDP-43 or the caspase-3 activation status in these mice was not studied.

Accumulation of another fragment with apparent molecular weight of ~35 kDa was also found in lymphoblastoid cell lines derived from patients with TDP-43 mutations (15,19), but in CNS tissues of patients, this 35 kDa fragment is either low or undetectable (6,54,55), suggesting that this fragment is not pathologic. Indeed, although a soluble 35 kDa fragment was detected in transgenic mice with wild-type TDP-43 overexpression at early stages, this fragment decreased with disease progression (222). The 35 kDa fragment may well represent a normal TDP-43 isoform generated from an alternative translation start site (204) and may play a physiological role in the formation of SGs (166,200,202,204).

### Ubiquitination, the ubiquitin-proteasome system and the autophagic system

The role of ubiquitination of TDP-43 in disease pathogenesis remains unknown, although it is likely to be a late event, since in patients the pre-inclusions and most TDP-43 inclusions are either weakly or not at all ubiquitinated (42,51,52). Nevertheless, extensive ubiquitination of the pathologic CTFs in cells (236) suggests that cellular degradation machineries such as the ubiquitin-proteasome system (UPS) and/or autophagy (reviewed in 246) may be involved in removing TDP-43 aggregates. Indeed, in cell culture systems, inhibition of either UPS or the autophagic system was shown to increase cytoplasmic TDP-43 accumulation and the formation of intracellular aggregates (202,231,239,247–249). Recently, TDP-43 was proposed to associate with ubiquitin 1 (UBQLN) (250), a protein that interacts with ubiquitinated proteins and was previously shown to play a role in Alzheimer's disease (reviewed in 251). UBQLN was found to potentiate TDP-43 autophagosomal degradation in cultured cells (250), and in *Drosophila*, the co-expression of UBQLN reduced TDP-43 levels but worsened the TDP-43-dependent phenotypes (220).

TDP-43 was also shown to affect the expression of histone deacetylase 6 (HDAC6), another protein associated with autophagosomal degradation (214). HDAC6 promotes the aggregation of polyubiquitinated proteins (252,253) and their autophagic degradation (254,255). A reduction in TDP-43 led to decreased HDAC6 levels resulting in reduced aggregate formation and cell death in a cellular model of spinocerebellar

ataxia (214). Genetic ablation of HDAC6 resulted in ubiquitinated protein aggregate accumulation and neurodegeneration in *Drosophila* and in mice (256). Interestingly, VCP shown to antagonize the HDAC6-dependent aggregation of ubiquitinated proteins (253) is mutated in patients with familial forms of FTD associated with inclusion body myopathy and Paget's disease (257). Moreover, ubiquitinated inclusions in brain and muscle of patients with VCP mutations (6,49,97,115) (Table 1) and of transgenic mice expressing mutant human VCP (258) contain TDP-43. VCP is implicated in multiple steps of degradation of misfolded proteins (reviewed in 259) including a critical role in autophagy, and pathological mutant VCP expression results in autophagic dysfunction in cells and in tissues from patients and transgenic mice (247,260–263), deficits which can be rescued by HDAC6 overexpression in cell culture (260).

Although the interconnections of the different players described above are yet to be established, the autophagic system is likely to play a crucial role in the pathogenesis of neurodegenerative disorders, as suggested by the discovery that the neuronal-specific deletion of genes essential for autophagy leads to accumulation of ubiquitinated protein aggregates and neurodegeneration in mice (264,265).

### Loss of TDP-43 nuclear function

The observation that the majority of inclusion-bearing cells in patients display nuclei devoid of TDP-43 led to the hypothesis that some of the deleterious effects of abnormal TDP-43 metabolism may reflect a loss of TDP-43 nuclear function (6,46). Indeed, expression of predominantly cytoplasmic TDP-43—with mutations that disrupt its NLS—led to sequestration of endogenous nuclear TDP-43 in cell culture (230). Furthermore, in transgenic mice expressing mutant human TDP-43, affected neurons displayed nuclei cleared of endogenous TDP-43, despite lack of TDP-43 immunoreactivity of cytoplasmic ubiquitinated inclusions (228). Although the first two sets of heterozygous mice with the *Tardbp* gene disruption showed no abnormalities (209,210), a most recent study demonstrated motor dysfunctions in aged heterozygous *Tardbp*<sup>+/-</sup> mice in the absence of neurodegeneration, despite lack of detectable reduction in TDP-43 protein levels (211). Similarly, TDP-43 loss can drive motor dysfunction in *Drosophila* (193). The contribution of disease-causing mutations in this possible nuclear loss of function mechanism remains unclear; at least in cell culture, they do not seem to affect the exon-skipping activity of TDP-43 (136,231), or the transcriptional regulation of HDAC6 (214), nor do they alter its protein interaction profile (166).

Although a direct test of an involvement of loss of TDP-43 or FUS/TLS function in disease pathogenesis is still missing, given the known physiological roles of these proteins, it is easy to speculate that their loss of function may have profound effects on RNA processing with detrimental consequences for the cell.

### CONCLUSIONS AND OPEN QUESTIONS

In conclusion, the weight of the evidence strongly implicates TDP-43 and FUS/TLS in neurodegeneration through errors

in multiple steps of RNA processing. Elucidating the physiological roles of these two proteins within the normal CNS is an essential first step in deciphering disease pathways. The major questions underlying pathogenesis remain unresolved: is disease from TDP-43 or FUS/TLS mutation caused by the loss of normal function, gain of one or more toxic properties or aberrant function or both? Post-translational modifications and cleavage of TDP-43 are markers for the disease state, but a pathogenic role of such alterations has not been established. Going forward, exploitation of advances in high-throughput sequencing is now needed to identify the normal RNA targets and the consequences of mutations on the processing of these RNAs. So too is improved modeling of disease in animal and cell culture systems so as to understand TDP-43- and FUS/TLS-mediated neurodegeneration.

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