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REVIEW

Proteomics elucidating physiological and pathological functions of TDP-43

Jorge García Morato[1] | Christian Johannes Gloeckner[2,3] | Philipp J. Kahle[1,4]

[1]Laboratory of Functional Neurogenetics, Department of Neurodegeneration, German Center of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany

[2]Research Group Functional Neuroproteomics, German Center of Neurodegenerative Diseases, Tübingen, Germany

[3]Core Facility for Medical Bioanalytics, Institute for Ophthalmic Research, Center for Ophthalmology, University of Tübingen, Tübingen, Germany

[4]Department of Biochemistry, University of Tübingen, Tübingen, Germany

Correspondence
Philipp J. Kahle, Laboratory of Functional Neurogenetics, Department of Neurodegeneration, German Center of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research, University of Tübingen, Otfried Müller Str. 27, 72076 Tübingen, Germany.
Email: philipp.kahle@uni-tuebingen.de

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Abstract

Trans-activation response DNA binding protein of 43 kDa (TDP-43) regulates a great variety of cellular processes in the nucleus and cytosol. In addition, a defined subset of neurodegenerative diseases is characterized by nuclear depletion of TDP-43 as well as cytosolic mislocalization and aggregation. To perform its diverse functions TDP-43 can associate with different ribonucleoprotein complexes. Combined with transcriptomics, MS interactome studies have unveiled associations between TDP-43 and the spliceosome machinery, polysomes and RNA granules. Moreover, the highly dynamic, low-valency interactions regulated by its low-complexity domain calls for innovative proximity labeling methodologies. In addition to protein partners, the analysis of post-translational modifications showed that they may play a role in the nucleocytoplasmic shuttling, RNA binding, liquid-liquid phase separation and protein aggregation of TDP-43. Here we review the various TDP-43 ribonucleoprotein complexes characterized so far, how they contribute to the diverse functions of TDP-43, and roles of post-translational modifications. Further understanding of the fluid dynamic properties of TDP-43 in ribonucleoprotein complexes, RNA granules, and self-assemblies will advance the understanding of RNA processing in cells and perhaps help to develop novel therapeutic approaches for TDPopathies.

KEYWORDS
interactome, liquid-liquid phase separation, RNA processing, TDP-43

Abbreviations: ALS, amyotrophic lateral sclerosis; CDC7, cell division cycle 7-related protein kinase; CK1, casein kinase 1; CTF, C-terminal fragment; FMRP, fragile X mental retardation protein; FTLD, frontotemporal lobar degeneration; FUS, fused-in-sarcoma; G3BP, GTPase-activating protein binding protein; HDAC, histone deacetylase; hnRNP, heterogeneous nuclear ribonucleoprotein; LLPS, liquid-liquid phase separation; lncRNA, long non-coding RNA; LSm, like Smith antigen; MALAT1, metastasis associated lung adenocarcinoma transcript 1; miRNA, microRNA; NEAT, noncoding nuclear-enriched abundant transcript; NLS, nuclear localization sequence; NUP62, nuclear pore glycoprotein of 62 kDa; Pat, protein associated with topoisomerase II; pre-miRNA, precursor miRNA; pri-miRNA, primary miRNA; SG, stress granule; snRNP, small nuclear ribonucleoprotein; STAU1, Staufen homolog 1; SUMO, small ubiquitin-related modifier; TDP-43, trans-activation response DNA binding protein of 43 kDa; TIA-1, T cell restricted intracellular antigen-1; TTBK, tau tubulin kinase; UTR, untranslated region.

1 | INTRODUCTION

The trans-activation response DNA binding protein of 43 kDa (TDP-43) is a nucleic acid binding protein with many diverse functions. Although originally cloned as a protein that binds to the trans-activation response DNA element within the human immunodeficiency virus long terminal repeat [1], very few DNA-binding functions have been described for TDP-43. For example, TDP-43 binds to the *SP-10* insulator, acting as a repressor for this acrosomal gene product in somatic tissues [2]. TDP-43 is enriched in euchromatin domains where transcriptional events take place [3], but the mechanisms by which TDP-43 regulates global transcription are poorly understood [4]. In cells exposed to genotoxic stress, TDP-43 was recruited to sites of DNA damage where it participated in non-homologous end joining DNA repair [5, 6]. Moreover, TDP-43 conferred DNA protection and genome stability by alleviating the co-transcriptional formation of R-loops that promote transcription/replication conflicts [7, 8].

Beyond DNA binding, far more studies established the RNA binding roles of TDP-43 (Figure 1). TDP-43 was initially discovered as a splicing factor mediating exon 9 exclusion of the cystic fibrosis transmembrane conductance regulator transcript [9]. By binding to mostly intronic GU-rich sequences, TDP-43 regulates splicing of canonical as well as cryptic exons [10–12]. By alternative splicing of cryptic exons TDP-43 regulates the use of alternative poly-adenylation sites, thus affecting the expression of its own transcript [13] or that of stathmin-2 [14]. In fact, TDP-43 binds to thousands of transcripts, not only pre-mRNAs (coding and non-coding) but also microRNA (miRNA) precursors [15, 16]. In addition to the regulation of alternative splicing, TDP-43 also mediates mRNA stability, as exemplified for the transcripts of neurofilament L [17] and the histone deacetylase (HDAC) 6 [18]. TDP-43 can form a complex with the fragile X mental retardation protein (FMRP) and the Staufen homolog STAU1, promoting stabilization of the HDAC1 transcript [19]. Moreover, TDP-43 can enhance mRNA translation via association with ribosomes [20]. Finally, TDP-43 participates in RNA transport processes in axons [21] and during the assembly of stress granules (SGs) [22].

The manyfold diverse functions of TDP-43 in all these steps of RNA processing are not only fascinating from a basic cell biology point of view, but also bear great disease relevance. Neuropathological TDP-43 inclusions [23, 24] constitute defining lesions of specific subtypes of the neurodegenerative dementia frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS), a motoneuron degeneration [25]. Moreover, mutations in the TDP-43 gene are linked to ALS [26–29]. During pathogenesis, TDP-43 must partition from its physiological complex sites to phase-separate into liquid droplets and further solidify into pathological aggregates. The challenge is to identify all these physiological complex compositions and the assemblies formed in disease course. Another essential issue is the regulation of TDP-43 targeting into the respective protein complexes, possibly involving post-translational modifications. Proteomic investigations continue to provide insight into this important topic.

2 | PROTEOMIC METHODOLOGIES TO STUDY TDP-43 COMPLEXES

As the majority of proteins do not act as single element but rather carry out distinct cellular functions in form of cell-type and context-specific protein complexes, the systematic dissection of protein-protein interaction networks gained importance in the past decades. In particular, the availability of fast and sensitive mass spectrometers fueled this process. The classical way to analyze protein complexes is the co-purification of interacting proteins with a bait protein, either by the recombinant expression of tagged proteins or IP using primary antibodies against the target protein. The precipitated proteins are then analyzed by MS. Originally tandem-affinity purification has been used to get highly pure protein complexes allowing to identify specific interactomes. Initially used for the investigation of protein complexes in yeast, protocols have been developed for the analysis of interactomic networks in mammalian cells [30-33]. A tandem-affinity purification MS approach using an extended HIS-tag system in combination with a custom-made antibody has recently been applied to TDP-43 [34], revealing interactions related to protein stabilization and protein folding upon oxidative stress stimulation. In addition, pull-downs with a recombinant protein or protein domain have widely been used to study protein-protein interactions [35, 36]. However, one major drawback of these approaches is its bias for rather stable protein interactions, given stringent washing conditions. As an alternative strategy to cope with unspecific background quantitative MS, either label-free of label-based, has been established to identify specific interactions. SILAC has widely been used for this purpose. IP combined with SILAC and a knock-down control emerged as a powerful tool to investigate protein complexes at their native expression levels [37, 38]. Affinity based methods have also been applied to TDP-43, in particular to the TDP-25 fragment (see below), to assign interactomic networks [39]. Another study uncovered binding of TDP-43 and a related ALS-associated RNA-binding protein FUS (fused-in-sarcoma) to several factors important to DNA repair mechanisms by affinity proteomics [40]. Furthermore, an ALS-linked mutation in TDP-43 has recently been shown to alter protein interactions involved in the motor neuron response to oxidative stress [41]. One of the most comprehensive studies so far to elucidate TDP-43 complexes for wild-type and two disease-causing variants (A315T and M337V) was an IP-based approach following the recombinant expression of FLAG-tagged TDP-43 bait proteins. This study revealed to clusters of distinct interaction networks, a nuclear/splicing cluster and a cytoplasmic/translation cluster while no alterations by the disease-causing variants were observed, most likely as the identification of co-precipitated proteins was relying on a qualitative, identification-based approach, not considering quantitative changes of the interactome [42]. Finally, a study, combining tandem-affinity purification with SILAC-based quantitative proteomics not only revealed expected interactions with heterogeneous nuclear ribonucleoproteins (hnRNPs) but also identified components of the Drosha complexes in the TDP-43 interactome, which is consistent with roles for TDP-43 in both mRNA processing and miRNA biogenesis [43].

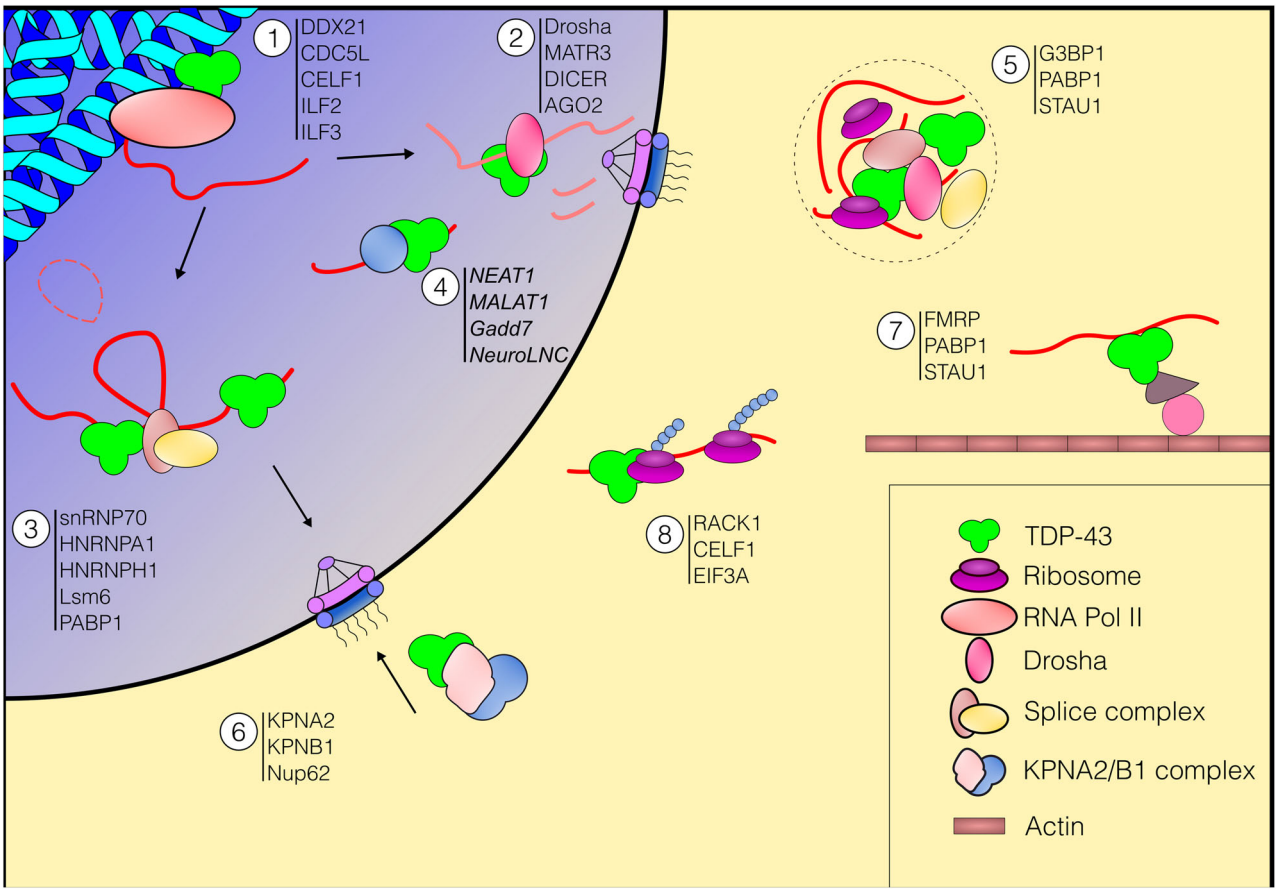

FIGURE 1 TDP-43 and main partners. While mostly nuclear, TDP-43 performs several vital functions both in the nucleus and in the cytoplasm of cells, interacting with distinct partners. The main functions are (1) RNA transcription, (2) miRNA processing, (3) mRNA splicing, (4) lncRNA processing, (5) stress granule formation, (6) nuclear-cytoplasmic shuttling, (7) mRNA transport, and (8) translation.

While these techniques certainly represent powerful tools to investigate large interactomic networks, they do not necessarily identify direct protein interactions. One method developed three decades ago is the yeast two hybrid system which emerged, thanks to the mating system, to a powerful screening tool of a large set of bait proteins [44]. By this approach, large protein interaction networks associated with neurodegenerative diseases have been described [45]. TDP-43 was one of the 500 bait proteins tested in this study. A more focused yeast two hybrid screen identified enzymes involved in the regulation of TDP-43 ubiquitinylation [46].

Despite of the significant achievements possible due to the systematic application of this methods, there is still an unmet need for the identification of weaker, transient interactions at physiological conditions and within intact cells. This is especially important for TDP-43 with its low-complexity domain that engages in fluid dynamic protein assemblies rather than rigid stable complexes. Recent developments of proximity-based methods allowing the direct or indirect proximity biotinylation of proteins in a considerable small distance to the bait protein might help to close this gap [47]. Proximity-dependent biotin identification involves bait protein fusion to enzymes based on bacterial BirA biotin ligases that have been subject of continuous optimization, now allowing a very efficient biotinylation at low biotin concentrations and in a short time. Alternatively, proximity labeling can be achieved by oxidative activation of biotin-phenol using ascorbate peroxidase. Both methods offer a great opportunity to gain more insight into the spatial organization of protein complexes orchestrated by TDP-43, in particular following specific interactions associated with RNA and/or liquid-liquid phase separation (LLPS). Consequently, a recent study has applied biotin proximity labeling to identify novel interaction partners of detergent-insoluble TDP-43 aggregates. The identified proximity-proteome was enriched for components of the nuclear pore complex and nucleocytoplasmic transport machinery which strongly implicate TDP-43-mediated nucleocytoplasmic transport defects as a common disease mechanism in ALS/FTLD [48]. The achievements of the interactomic studies and their impact on the current understanding of the TDP-43 (patho-)physiology is discussed in greater detail in the next section.

3 | TDP-43 INTERACTOMES

While unbiased approaches searching for TDP-43 partners have provided a wealth of information, we can now use these data to focus

TABLE 1 List of TDP-43 protein interactors detected in at least 2 independent studies, sorted by function in RNA processing.

mRNA splicing and nuclear export	
FUS/TLS	[17, 34, 40, 41, 84]
hnRNPA1	[36, 42, 43, 56]
hnRNPA2/B1	[19, 42, 43, 56]
hnRNPA3	[42, 43, 56]
hnRNPDL	[36, 43]
hnRNPH1	[36, 42, 43]
hnRNPK	[19, 42, 43]
hnRNPQ	[19, 42, 43]
hnRNPU	[36, 42, 43]
hnRNPUL1	[36, 42, 43]
hnRNPUL2	[36, 42, 43]
PTBP1	[42, 43, 117]
PABPC1	[42, 43, 187]
CELF1	[42, 43, 117]
snRNP70	[43, 58]
Matrin-3	[42, 43, 117, 188]
Cytosolic mRNA transport and translation control/SG	
STAU1	[19, 42, 189]
FMRP	[19, 190]
RACK1	[44, 101]
TIA1	[36, 189]
G3BP	[42, 88]
PABPC1	[42, 187]
miRNA processing	
Drosha	[16, 191]
DGCR8	[16, 191]
Dicer	[16, 192]
DDX17	[36, 42, 43]
Ago2/EIF2C2	[16, 42]
Transcription	
DDX3X	[36, 42, 43]
DDX5	[36, 42, 43, 193]
Nucleocytoplasmic shuttling	
KPNA2	[42, 121, 194]
NUP62	[126, 195]
KPNB1	[121, 126, 194]

on TDP-43 functions and attempt to describe in detail the nature of TDP-43 interactions in relation to the function and complex where it is found (Table 1). In the following sections this review will try to identify specific roles of TDP-43 in the different ribonucleoprotein complexes where it is found and describe the nature of its interactions there.

3.1 | miRNA processing

MiRNAs are small, non-coding RNAs with an average size of 22 nucleotides, which can regulate gene expression by affecting mRNA stability and protein expression. They can be encoded in intergenic regions of the genome or in introns of related or unrelated genes [49]. Most of the miRNA transcripts are produced as pri-miRNA, which is processed in the nucleus by the Drosha complex into pre-miRNA. After that, pre-miRNA is exported via the exportin 5 system to the cytoplasm [50], where it is further processed by the Dicer complex, producing mature miRNAs. This mature miRNA can then alter expression of its targets [50]. Interestingly, TDP-43 is involved in both the nuclear and cytoplasmic portions of the miRNA maturation process.

The clearest evidence that shows an involvement of TDP-43 in the miRNA maturation process is the direct interaction it has with Drosha, Dicer and argonaute-2 [16, 42]. TDP-43 can bind to both Drosha and Dicer via its C-terminal domain, and the Drosha interaction seemed to be affected by TDP-43 phosphorylation at S409/410 [16, 51]. A closer inspection of TDP-43 role in both complexes shows that it can directly bind to some pri-miRNAs via the consensus poly (UG) sequence, such as pri-miRNA-574, pri-miRNA-578 and increase the binding affinity of the Drosha complex to those sequences [16]. This data is supported by the fact that silencing TDP-43 triggers a nuclear accumulation of unprocessed pri-miRNA let-7b, pri-R-181c, pri-miRNA-574 and pri-miRNA-578 [15, 16, 52], suggesting that a lack of TDP-43 reduces the pri-miRNA cleavage efficiency of the Drosha complex. Because of this, it is not surprising that a reduction in TDP-43 levels can alter the levels of several miRNAs [52, 53].

Interestingly, some of the gene targets of these miRNAs have been found to be altered in TDP-43 depletion models, which offers a mechanism linking both datasets [15, 53]. To link these results to disease, the effect of TDP-43 on miRNA biogenesis has been investigated in differentiating neurons and in ALS and FTLD-TDP patients. A knockdown of TDP-43 in differentiating neurons causes reduction in Drosha levels, however Drosha mRNA levels do not change and the exact mechanism for this change is not clear [53]. In addition, several miRNAs have been reported to be altered in patients with TDP-43 pathology, but the disease relevance of this finding remains unknown [54]. Finally, it is worth noting that the results of the studies seem to be very cell-type specific and suggest a highly complex and dynamic regulation of miRNAs by TDP-43.

3.2 | RNA splicing

The splicing of thousands of RNAs have been linked to the presence of TDP-43, both in terms of exon splicing and in the suppression of cryptic exons [10–12]. The role of TDP-43 in splicing it is further supported by its interactions with several spliceosome and splicing-related proteins. For instance, TDP-43 has been found associated with proline/glutamine-rich and serine/arginine-rich splicing factors and many hnRNPs [42]. Indeed, TDP-43 functions in conjunction with hnRNPs to promote splicing [55–57].

More directly, TDP-43 interacts with the small nuclear ribonucleoprotein of 70 kDa (snRNP70) [58], a component of the U1 snRNP complex that stabilizes the interaction between the snRNA U1 and the 5′ splice site of a processed pre-mRNA [59]. TDP-43 was also detected by MS among the proteins co-immunoprecipitated with FLAG-tagged C9ORF78, a natively unfolded protein that acts within the U5 snRNP [60]. As the C9ORF78 interaction partner snRNP200/BRR2 helicase was enriched in TDP-43 IPs [42], integration of TDP-43 into the 3′ splice site helicase activity could in part explain the alternative splicing functions of TDP-43, in addition to a potential role for pre-mRNA 5′ splice initiation at the U1 snRNP complex.

"Like Smith antigen" (LSm)6 was identified as a TDP-43 partner in a yeast two-hybrid study [46]. LSm6 together with LSm5 and LSm7 organizes the formation of LSm heteroheptameric rings [61]. In the nucleus, the LSm2–8 complex is engaged in the assembly of the pre-catalytic spliceosomal B complex by binding to the 3′ end of U6 snRNA, and is part of the structural reorganizations that occur in the subsequent spliceosome activation step involving the aforementioned helicase snRNP200/BRR2 that unwinds the U4/U6 snRNAs [62]. Interestingly, there is a second LSm complex in the cytoplasm (LSm1–7) that is part of the mRNA degradation machinery [63–65]. Likewise, there are dual functions of the LSm associated "protein associated with topoisomerase II" (Pat)1b in the nucleus and in the cytoplasm [66]. Because TDP-43 can also shuttle between the nucleus and the cytoplasm, such interactions are particularly noteworthy. Moreover, Pat1 can enhance LLPS of mRNA decay factors in processing bodies [67, 68], membraneless organelles that are sites of mRNA degradation, storage and repression. Taken together, TDP-43 with its intrinsically disordered domains might be involved in structurally dynamic assemblies that mediate RNA splicing in the nucleus as well as mRNA regulation in RNA granules (see also below).

Coupling of RNA splicing and decay underlies the mechanism of TDP-43 autoregulation through a negative feedback loop [11, 69, 70]. TDP-43 autoregulation has been confirmed in vivo both in mouse models [71] and in an FTLD patient with a mutation in the 3′-untranslated region (UTR) of TDP-43 mRNA, resulting in higher TDP-43 levels [72]. TDP-43 binds to a conserved region in the 3′-UTR of its own transcript. The binding to this sequence promotes TDP-43 oligomerization and assembly into dynamic ribonucleoprotein granules [73]. Two autoregulation mechanisms involving alternative splicing have been described, one dependent on nonsense-mediated RNA decay and the other one exosome-dependent. In the nonsense-mediated decay model, TDP-43 binding favors alternative polyadenylation signals pA2 and pA4, leading to RNA degradation [70]. The second proposed mechanism involves inclusion of a cryptic exon 7. This alternatively spliced mRNA is highly unstable, and it is degraded via the exosome system [69, 74]. By a similar mechanism targeting a cryptic exon TDP-43 regulates expression of the autophagy gene product ATG4B [10, 75].

In addition to its own transcript, TDP-43 binds to 3′-UTR regions of many more mRNAs [12, 76, 77]. For example, TDP-43 binding to the 3′-UTR stabilizes the neurofilament L mRNA [17], which can be affected by TDP-43 mislocalization and relieved by autophagy induction [78]. Likewise, TDP-43 binds to the 3′-UTR of the transcript encoding GTPase-activating protein binding protein (G3BP) to promote its expression [79, 80]. Moreover, binding of TDP-43 within the coding region of HDAC6 mRNA stabilized its protein expression [18, 81]. On the other hand, overexpression of TDP-43 destabilized the mRNA coding for the FTLD-linked gene product progranulin and hence reduced its protein levels [77]. As this interaction was detected in the cytosol, TDP-43 affects mRNA levels not only at the level of alternative pre-mRNA splicing in the nucleus, but also controls mRNA stability in the cytosol.

3.3 | mRNA transport

After splicing, capping and polyadenylation, mature RNA is transported to the cytoplasm for further translation at the ribosome. Several RNA-binding proteins are known to play a role in this process, being TDP-43 one of them. While the mRNA transport takes place in every cell, in large cells with complex morphology such as neurons it is a critical process that allows for local translation of proteins that are needed at neurites or distal segment of the axons. It is in this context where TDP-43 has been found to interact with several other RNA-binding proteins and play an important role.

The previously mentioned serine/arginine-rich splicing factors [42] could couple splicing with nuclear export of mRNA [82]. Once in the cytosol of neurons, TDP-43 colocalizes with mRNA granules in axons [83], where it can interact with FMRP and STAU1 [19, 42, 84]. A closer look at these interactions showed that TDP-43 can mediate both anterograde and retrograde transport depending on the protein partner. TDP-43 is engaged in anterograde transport when interacting with FMRP, while interaction with STAU1 mediates retrograde transport [84]. Interestingly, pathological mutants M337V and A315T impaired RNA granule transport along axons [21]. Since both of these mutations are located in the C-terminal domain (Figure 2A), linked with protein-protein interaction, it is possible that the defect in mRNA transport comes not from a change in mRNA binding affinity, as much as a change in protein binding affinity to other partners, such as FMRP and STAU1. Specifically, substitutions at W334 in TDP-43 C-terminal low-complexity domain impact both the number of mRNA granules found in axons and mRNA anterograde transport [85].

When involved in axonal transport, TDP-43 forms liquid droplets that, interestingly, change their biophysical properties as they circulate along the axon [86]. As TDP-43 granules move from proximal to distal sections of the axons the liquid phase becomes more fluid and dynamic. This transition is affected by pathological mutations (M337V and G298S). The reasons behind this transition and its significance in TDP-43 pathophysiology remains to be uncovered.

3.4 | Stress granules

In addition to processing bodies and transport granules (see above), cytosolic mRNA can also be packaged into SGs [87], along with prominent recruitment of TDP-43 [22]. SGs are membrane-less organelles

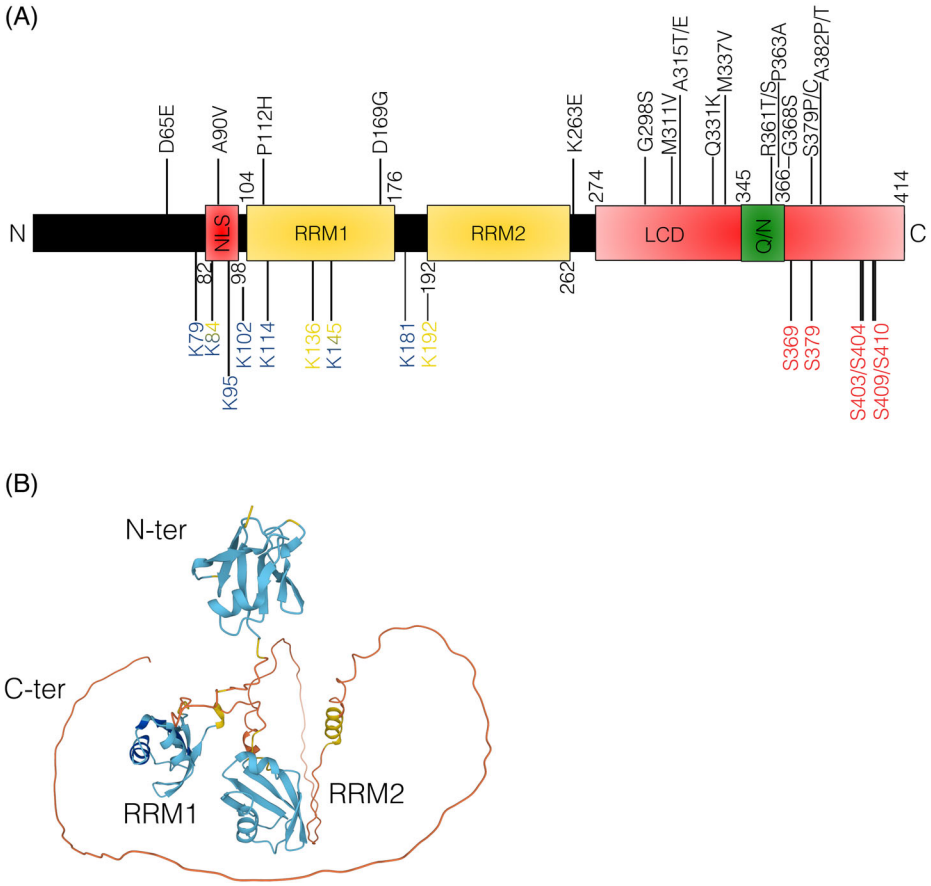

FIGURE 2 Structure and domains of TDP-43. A) TDP-43 is composed by a well-structured N-terminal region, containing a nuclear localization sequence (NLS) and two RNA recognition motifs (RRM1 and RRM2). The low complexity domain at the C-terminal part contains a glutamine/asparagine-rich region (Q/N). In the upper part of the image, the most prominent pathogenic mutations are annotated. In the lower part of the image the post-translational modifications that are referred to in the text are color-coded. Blue, yellow and red stand for ubiquitination, acetylation and phosphorylation, respectively. Residues with more than one annotated PTM are marked with two colors. B) Structural model of TDP-43 provided by AlphaFold [186]. Protein domains are annotated, including the disordered C-terminal tail.

that form upon different cell stressors, such as oxidative stress or heat shock. They are formed by an inner stable core and a fluid shell, and they are composed by a mixture of RNAs, RNA-binding proteins, ribosomes and scaffold proteins [88]. Their canonical function is to store stalled ribosomes during stress and continue translation after the stress has disappeared, however this hypothesis has been questioned in recent years. While translational repression elements are enriched in SGs [89], they are not entirely essential for translational repression as single molecule imaging has revealed active translation within SGs [90]. It is at present unclear what the ultimate function of these organelles are, or even if they are a mere epiphenomenon triggered by stress without a specific function [91].

Over 200 proteins have been found to be enriched in SGs from stressed cells, and several of those, such as G3BP and T cell restricted intracellular antigen-1 (TIA-1), have been identified as members of the SG core [88]. Interestingly, TDP-43 has been found to interact with both of them in an RNA-independent manner [92] and it is recruited to SGs upon cell stress [22, 80]. However, the role of TDP-43 in SGs is less clear. Knockdown of TDP-43 causes a reduction in G3BP mRNA and protein levels, while on the other hand the same depletion can increase TIA-1 amounts in cells [80, 93]. These modulations of SG core components do not prevent their formation, but they do change the engagement and disengagement dynamics. A closer look at the interaction between G3BP and TDP-43 shows that upon artificial G3BP SG formation via an optogenetic system, TDP-43 and TIA-1 are recruited to the initial G3BP1 granules [94]. These results support the idea that G3BP is an early core element of SGs and that TDP-43 is perhaps a recruiter of RNA targets to SGs. The impact in SGs dynamics caused by TDP-43 knockdown could be due to the modulation of SG members' mRNAs, and not via direct action of TDP-43 at the SGs. In SG-inducing cellular stress conditions TDP-43 still forms insoluble aggregates when SG formation proper is blocked [95, 96]. Thus, although TDP-43 clearly participates in SGs, the (patho)physiological significance remains obscure.

3.5 | Protein translation

Beyond translational stalling in SGs TDP-43 may generally affect protein translation via a cytosolic translation interactome cluster [42].

Particularly when the predominantly nuclear TDP-43 is mislocalized in the cytoplasm it can cause a decrease in global protein synthesis [97, 98]. Overexpression of TDP-43 did not seem to cause changes in total translation but rather affected translation of select neurodegeneration-relevant mRNAs identified by ribosome profiling [20]. TDP-43 binding to such mRNAs caused altered translation in overexpression or cytoplasmic mislocalization models [99–101]. Association of TDP-43 with polysomes [20, 98] may not only occur by binding to RNA but also proteins, such as the receptor for activated C kinase 1 [42, 46, 98], a multifunctional scaffold protein present in ribosomes. Also, TDP-43 mediated alternative splicing of the exon junction complex protein ribosomal S6 kinase 1 Aly/REF-like target (also known as polymerase delta interacting protein 3) has been shown to affect global translational yield [102]. Moreover, TDP-43 is involved in the axonal transport of ribosomal proteins and reduced levels of TDP-43 can cause a reduction in local translation in the axons [100, 103]. Thus, in addition to its functions in RNA processing, TDP-43 may also affect the (patho)physiological proteome via protein translation regulation [104].

3.6 | Non-coding RNAs

In addition to the RNA species mentioned above, TDP-43 also interacts with long non-coding RNAs (lncRNAs) [12]. The level of metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*) (aka noncoding nuclear-enriched abundant transcript 2, *NEAT2*) is regulated by TDP-43, with overexpression of TDP-43 causing an increase in MALAT1 RNA levels, and TDP-43 knockdown causing the opposite [105]. A detail examination of the *MALAT1* elements identified a short interspersed nuclear element to be involved in the localization of the molecule, whose deletion would cause mislocalization to the cytoplasm and sequester TDP-43 into liquid droplets [106].

In the context of *NEAT1* interaction, it has been reported that TDP-43 can colocalize with it in paraspeckles [107]. Specifically, the isoform *NEAT1_2* seems to act as a scaffold and recruits TDP-43 into these nuclear bodies, triggering TDP-43 LLPS [108, 109]. Interestingly, the disease-linked mutation D169G interfered with the *NEAT1*-mediated TDP-43 LLPS [109]. On the other hand, silencing of TDP-43 increased levels of the stress-induced long isoform *NEAT1_2* and stimulated paraspeckle assembly [110, 111]. Conversely, upon accumulation of TDP-43 the major long isoform *NEAT1_1* was found up-regulated and appeared to counteract pathological effects of TDP-43 [112]. TDP-43 interplay with *NEAT1* therefore seems to be an important regulatory mechanism influencing cellular viability via paraspeckles, nuclear domains mediating RNA processing and metabolism [113].

MALAT1 and *NEAT1* are among the strongest TDP-43 binding RNAs [12]. In addition, a lncRNA called growth-arrested DNA damage-inducible gene 7 was found to compete with TDP-43 binding to the mRNA encoding cyclin-dependent kinase 6, thereby interfering with its mRNA decay and thus controlling cell cycle progression in Chinese hamster ovary cells [114]. Binding of TDP-43 to *neuroLNC* in this case selectively stabilized mRNAs encoding synaptic vesicle proteins to ensure presynaptic function and neuronal excitability [115]. Last but not least, SILAC profiling found TDP-43 enriched in chromatin with the X-inactive specific transcript [116] where it participates in a condensate required for gene silencing [117]. The relevance of specific TDP-43 interacting hnRNPs in this process [118] remains to be elucidated. It emerges that TDP-43 regulates cellular proteomes not only by interactions with and partitioning of protein-coding mRNAs and miRNA processing, but also via lncRNAs.

3.7 | Nucleocytoplasmic shuttling

The manyfold functions of TDP-43 in the nucleus and cytoplasm obviously require nucleocytoplasmic shuttling, which is mediated by active nuclear import through a bipartite nuclear localization sequence (NLS) between K82-K98 and probably passive, exportin-independent nuclear export [119, 120]. Importin-α1 recognizes the TDP-43 NLS and forms a heteromeric importin-α1/β complex with TDP-43, and this interaction can be modulated by three phosphorylations at the NLS (T88, S91 and S92) [121]. In addition, the key NLS residue K84 can be ubiquitinated as well as acetylated, with both modifications altering TDP-43 nucleocytoplasmic shuttling [122, 123] (see below). In FTLD and ALS this balance is altered and cytoplasmic mislocalization of TDP-43 is considered a clear marker of its proteinopathy [23, 24]. Because of this, it has been the focus of a considerable amount of research.

The deletion mutant ΔNLS-TDP-43 mislocalizes to the cytoplasm, where it can become less soluble [124]. In addition, under arsenite stress TDP-43 shifts into the cytoplasm where it may become less soluble and phase-separates into SGs and eventually turns into protein inclusions similar to those found in patients [125]. In addition, cytoplasmic aggregates of C-terminal truncated TDP-43 can recruit nuclear import elements such as importin-α and nuclear pore components such as the nuclear pore glycoprotein of 62 kDa (NUP62), disrupting general nuclear import and the nuclear lamina structure [48, 125]. Interestingly, NUP62 co-aggregated with cytoplasmic TDP-43 can recruit karyopherin-β1, which in turn interacts with aggregated TDP-43 C-terminal fragment (CTF) to reduce aggregation [126]. This newly reported interaction hints to a complex network of interactions between TDP-43 and nuclear transport elements that is not limited to the importin system and opens new ways to reduce TDP-43 aggregation.

4 | POST-TRANSLATIONAL MODIFICATIONS OF TDP-43

It was evident already at the time of discovery that TDP-43 in disease is altered by modifications including phosphorylation, ubiquitinylation, and truncation leading to CTFs commonly referred to according to their approximate molecular masses as TDP-35 and TDP-25 [23, 24]. Moreover, under oxidative stress TDP-43 cysteine residues C173-

C175 can form disulfide bonds [127] and in the concomitant presence of nitric oxide these residues become S-nitrosylated [128], leading to oxidation-mediated TDP-43 aggregation and neuropathology. Thus, the investigation of TDP-43 PTMs may provide important clues for the regulation of (patho)physiological functions and disease-promoting processing of TDP-43.

4.1 | TDP-43 phosphorylations

The most commonly detected pathological modification of TDP-43 is phosphorylation of serines −409 and −410 [129, 130] (Figure 2A). It is of note that although potential phospho-acceptor threonine and serine residues are spread across the entire sequence of TDP-43, immunoreactivity with post-mortem disease tissue was only detected with antibodies selective for serine residues in the C-terminus [131, 132]. Consistently, phospho-sites were clustered in the TDP-43 C-terminus in a small LC-MS analysis of insoluble material extracted from the brains of 2 ALS patients [133]. Similar to other intracellular amyloidogenic proteins [134, 135], the broadly acting casein kinases were capable of phosphorylating TDP-43 in vitro. Casein kinase 1 (CK1) strongly phosphorylated TDP-43 at serines 379, 403/404, and 409/410 and appeared to promote TDP-43 aggregation in vitro [131]. The CK1ε homolog *doubletime* in Drosophila enhanced TDP-43 toxicity in a fly model [136]. Expression of a constitutively active form of CK1δ promoted TDP-43 pathology in SH-SY5Y neuroblastoma cells [137]. Endoplasmic reticulum stress might be a trigger for CK1-dependent TDP-43 phosphorylation and aggregation in motor neuron-like cells [138]. Inhibition of CK1δ showed protective effects in [A315T]TDP-43 transgenic mice and in lymphoblasts of ALS patients [139]. However, Gruijs da Silva et al. recently reported on the contrary that CK1δ phosphorylation as well as phosphomimic mutagenesis did not affect TDP-43 functions but rather suppressed TDP-43 aggregation, rendering TDP-43 condensates more liquid-like and dynamic [39]. It remains to be further established if CK1 inhibitors are a viable therapeutic option for the treatment of ALS [140].

Screening for TDP-43 kinases in *C. elegans*, Liatchko et al. found cell division cycle 7-related protein kinase (CDC7) to phosphorylate TDP-43 S409/410 and promote toxicity in worms [141]. CDC7 inhibition decreased TDP-43 phosphorylation in a variety of models and restored TDP-43 function in human patient lymphoblasts [142, 143]. Moreover, the tau-tubulin kinases 1 and 2 (TTBK1/2) were identified as putative disease promoting pS409/410 TDP-43 kinases [144]. Unlike other putative TDP-43 kinases, TTBK1 is expressed predominantly in the CNS and thus localized to neurodegeneration-relevant areas [145]. TTBK1 was confirmed to phosphorylate TDP-43 at disease-relevant sites S409/410 and S403/404 in vitro and in arsenite-stressed cells and to induce pathological TDP-43 effects including cytoplasmic mislocalization [146]. As more effective TTBK1 inhibitors are being developed [147], it will become interesting to explore the therapeutic potential and relative contribution of TTBK1 and 2 for FTLD-TDP and FTLD-TAU [148].

4.2 | TDP-43 ubiquitinylations and SUMOylations

The attachment of ubiquitin moieties in post-mortem brain tissue of TDPopathy patients was evident already from the first study [23]. Formation of diverse ubiquitin chains on lysine residues of target proteins are most important PTMs that regulate a large variety of cellular fates, including protein trafficking and turnover [149]. Specifically, the attachment of poly-ubiquitin chains linked via lysine-48 targets proteins to proteasomal breakdown while ubiquitin binding motifs couple ubiquitinylated cargo to the autophagy machinery. Thus, initial studies dealt with the question of proteasomal and autophagic breakdown of TDP-43. Cells treated with proteasome inhibitor showed accumulation of poly-ubiquitinylated, insoluble TDP-43 [124, 150]. The proteasome appears to act synergistically with the autophagy machinery in the catabolism of aggregating TDP-43 [151, 152].

MS analyses assessing the characteristic DiGly-shifts indicated several lysine residues within TDP-43 as putative ubiquitin anchor sites [123, 153]. Site-directed mutagenesis revealed not a single lysine residue to account for TDP-43 ubiquitinylation effects, indicating considerable redundancy of the TDP-43 lysine-ubiquitin system. The RNA binding region harbored one cluster of ubiquitinylated TDP-43 lysine residues (K102, K114, K145, K181) [153], which were also detected in global ubiquitylome surveys [154, 155]. Explicit investigation of DiGly motifs in pulled-down TDP-43 once more confirmed ubiquitinylation at K181, which appeared constitutive in all conditions [123]. Interestingly, a second cluster of putative TDP-43 ubiquitinylation sites was detected after proteasome inhibition at the NLS residue K84 and K95 [123], as well as K160 that had been previously found in proteome-wide screens of ubiquitinylation sites of proteasome-inhibited cells [154, 155]. Site-directed mutagenesis confirmed K95 as a potential proteasome-targeting ubiquitinylated residue [123] and was a major residue mediating TDP-43 mislocalization upon proteasome inhibition by administration of poly-GA protein [156] that can be produced from pathogenic repeat expansions of the FTLD/ALS gene *C9ORF72*.

Taken together, TDP-43 can be ubiquitinylated at several lysine residues (Figure 2A), and not a single site confers proteasome or autophagy targeting except for possibly K95 under special conditions. Moreover, any influences of non-classical ubiquitinylations on RNA binding, subcellular localization, LLPS and aggregation remain to be further explored. It is noteworthy that ubiquitinylated TDP-43 tends to be shifted into mislocalized insoluble aggregates, for example after expression of the ubiquitin ligase parkin [157], UBE2E ubiquitin-conjugating enzymes [46], the von Hippel–Lindau cullin-2 substrate binding component [158] or FTLD/ALS-linked mutant cyclin F dependent ubiquitin ligase complex [159]. Conversely, the zinc finger protein 179 [160] and the ubiquitin ligase Praja1 [161] enhanced ubiquitin-dependent clearance of TDP-43. Clearly ubiquitinylations of TDP-43 are highly complex and the outcomes likely depend on cellular context and ubiquitin code written be distinct ubiquitin ligases. As for de-ubiquitinating enzymes, only the rather general ubiquitin isopeptidase Y has been described so far, counteracting ubiquitinylated TDP-43 pathology in cell and fly models [46].

TDP-43 is also a target for the small ubiquitin-related modifier (SUMO) [162, 163]. A SUMOylation consensus site comprises K136 within the RNA binding domain. Inhibition of SUMOylation with anacardic acid treatment of K136R substitution reduced TDP-43 aggregation and cytotoxicity [162]. Mutant [K136R]TDP-43 showed reduced binding and splicing activity towards several target mRNAs [163]. However, it should be noted that K136 is also subject to lysine acetylation (see below), so it has to be carefully determined if SUMOylation or acetylation account for effects observed by K136 mutagenesis.

4.3 | TDP-43 lysine acetylations

In addition to ubiquitin modifications, protein lysine residues can also be acetylated (Figure 2A). TDP-43 acetylation at K145 and K192 was first described by Cohen et al. [164]. It was found that stress-induced K145 modification directly alters RNA binding activities and aggregation propensity of TDP-43. Histone deacetylase 6 removed this potentially pathogenic modification. Similar effects were found for TDP-43 K136 acetylation, in this case sirtuin-1 acted as a relieving deacetylase [122]. Importantly, in this study amber suppression expansion of the genetic code was established for the first time to introduce the authentic modified amino acid into defined sites. Although site-directed mutagenesis is a powerful tool to stimulate small post-translational modifications of amino acids such as serine/threonine phosphorylation and lysine acetylation, respectively, the aspartate/glutamate and glutamine substitutions are not identical to the phosphorylated or acetylated residues. Although molecular dynamics simulations suggested little impact of the K136R substitution on the local structure within the RNA-binding domain [163], subtle clashes with bound RNA cannot be ruled out [122]. Nevertheless, amber suppression confirmed the results of site-directed mutagenesis, showing that K136 acetylation reduced RNA binding and splicing activity. Importantly, the K145 or K136 acetylation-mediated disengagement of TDP-43 from native RNA-binding protein complexes led to LLPS and subsequent pathological aggregation of TDP-43 in the nucleus, or when combined with nuclear import deficiency, formation of modified TDP-43 inclusions in the cytosol, the most common hallmark of TDPopathies [122, 165]. Demixing of RNA binding deficient TDP-43 into liquid droplets and conversion into gel/solid is controlled by chaperone proteins, such as HSP70 in the nucleus [166] or HSPB1 in the cytosol [167], likely as part of an HSF1-induced chaperone response system [165].

Moreover, K84 was identified as another acetylated residue in TDP-43. As this is a key determinant of the nuclear import sequence [123, 124], K84 acetylation affected the nucleocytoplasmic distribution of TDP-43 [122]. Taken together, it is noteworthy that similar to the lysine ubiquitin modifications also the acetylated lysine residues are primarily located in the nuclear import sequence and the RNA binding domain. These essential aspects of TDP-43 (patho)biology are at least in part regulated by lysine modifications, with acetylations in the RNA-binding region (Figure 2B) apparently having the most straightforward pathogenic potential [168].

4.4 | TDP-43 truncations

The presence of TDP-43 CTFs is a conspicuous feature discovered in FTLD and ALS patient brain [23, 24] but not as prominent in spinal cord [41, 169]. There is considerable CTF heterogeneity in patient isolates [133, 170, 171], making it hard to pinpoint a defined protease cleavage mechanism. Nonaka et al. found putative cleavage sites between M218-D219 and E246-D247 in a MS analysis of insoluble TDP-43 from FTLD brain and reported that such transfected CTF-GFP fusion proteins caused aggregation and interfered with TDP-43 splicing activity in neuroblastoma SH-SY5Y cells [172]. Caspases can cleave TDP-43 to roughly appropriately sized CTFs [173–176]. Although apoptotic caspase stimulation caused some CTF formation it did not appear to be an indispensable factor for TDP-43 aggregation and cytotoxicity and might even protect against full-length TDP-43 pathology [177, 178]. It has to be noted that CTFs may not only be formed by post-translational proteolytic processing, but can also arise from alternative splicing [179, 180]. Less is known about the N-terminal fragment counterparts, which appear to be more short-lived [181] but do have aggregation propensity [182, 183]. While it is clear that truncated TDP-43 species exist, their production mechanisms and (patho)physiological relevance needs to be further established [184, 185].

5 | CONCLUSIONS AND OUTLOOK

TDP-43 is a multifaceted protein with crucial roles RNA translation and processing and, in addition, it is the main component of protein aggregates in both ALS and FTLD-TDP. The knowledge about TDP-43 pathophysiology has exploded in the years after its identification as a pathological marker of these diseases, but even now it is challenging to establish the effect of TDP-43 dysfunction in the different cellular loci where it is involved. Traditional interaction studies identified several TDP-43 partners and thus helped understanding some of its functions. New techniques such as proximity labeling are now able to identify partners in highly dynamic contexts. The proliferation of such studies, in combination with the already published interactome studies, will expand enormously our knowledge of membrane-less organelles and identify how TDP-43 impacts the dynamics of RNA granules.

In addition to proximity labeling, the knowledge about protein structures and molecular grammar of low valency interactions have been expanded to a point where we can predict and to a certain extent influence LLPS in cells. Future studies could establish how different membrane-less organelles are organized, and how the interaction of their components can alter the physical characteristics. The understanding of this process could prove extremely valuable for the description of the molecular causes of TDP-43 proteinopathies and could open the door to new therapies and diagnostic tools.

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CONFLICT OF INTEREST STATEMENT

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Data sharing is not applicable to this article as no new data were created for in this literature review.

ORCID

Philipp J. Kahle https://orcid.org/0000-0002-8587-1731

REFERENCES

1. Ou, S. H., Wu, F., Harrich, D., García-Martínez, L. F., & Gaynor, R. B. (1995). Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. *Journal of Virology*, *69*, 3584–3596.
2. Abhyankar, M. M., Urekar, C., & Reddi, P. P. (2007). A Novel CpG-free vertebrate insulator silences the testis-specific SP-10 gene in somatic tissues. *Journal of Biological Chemistry*, *282*, 36143–36154.
3. Casafont, I., Bengoechea, R., Tapia, O., Berciano, M. T., & Lafarga, M. (2009). TDP-43 localizes in mRNA transcription and processing sites in mammalian neurons. *Journal of Structural Biology*, *167*, 235–241.
4. Morera, A. A., Ahmed, N. S., & Schwartz, J. C. (2019). TDP-43 regulates transcription at protein-coding genes and Alu retrotransposons. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, *1862*, 194434.
5. Konopka, A., Whelan, D. R., Jamali, M. S., Perri, E., Shahheydari, H., Toth, R. P., Parakh, S., Robinson, T., Cheong, A., Mehta, P., Vidal, M., Ragagnin, A. M. G., Khizhnyak, I., Jagaraj, C. J., Galper, J., Grima, N., Deva, A., Shadfar, S., Nicholson, G. A., … Atkin, J. D. (2020). Impaired NHEJ repair in amyotrophic lateral sclerosis is associated with TDP-43 mutations. *Molecular Neurodegeneration*, *15*, 51.
6. Mitra, J., Guerrero, E. N., Hegde, P. M., Liachko, N. F., Wang, H., Vasquez, V., Gao, J., Pandey, A., Taylor, J. P., Kraemer, B. C., Wu, P., Boldogh, I., Garruto, R. M., Mitra, S., Rao, K. S., & Hegde, M. L. (2019). Motor neuron disease-associated loss of nuclear TDP-43 is linked to DNA double-strand break repair defects. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 4696–4705.
7. Giannini, M., Bayona-Feliu, A., Sproviero, D., Barroso, S. I., Cereda, C., & Aguilera, A. (2020). TDP-43 mutations link Amyotrophic Lateral Sclerosis with R-loop homeostasis and R loop-mediated DNA damage. *PLoS Genetics*, *16*, e1009260.
8. Wood, M., Quinet, A., Lin, Y. L., Davis, A. A., Pasero, P., Ayala, Y. M., & Vindigni, A. (2020). TDP-43 dysfunction results in R-loop accumulation and DNA replication defects. *Journal of Cell Science*, *133*, jcs244129.
9. Buratti, E., & Baralle, F. E. (2001). Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of *CFTR* exon 9. *Journal of Biological Chemistry*, *276*, 36337–36343.
10. Ling, J. P., Pletnikova, O., Troncoso, J. C., & Wong, P. C. (2015). TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. *Science*, *349*, 650–655.
11. Polymenidou, M., Lagier-Tourenne, C., Hutt, K. R., Huelga, S. C., Moran, J., Liang, T. Y., Ling, S.-C., Sun, E., Wancewicz, E., Mazur, C., Kordasiewicz, H., Sedaghat, Y., Donohue, J. P., Shiue, L., Bennett, C. F., Yeo, G. W., & Cleveland, D. W. (2011). Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nature Neuroscience*, *14*, 459–468.
12. Tollervey, J. R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., König, J., Hortobágyi, T., Nishimura, A. L., Zupunski, V., Patani, R., Chandran, S., Rot, G., Zupan, B., Shaw, C. E., & Ule, J. (2011). Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nature Neuroscience*, *14*, 452–458.
13. Avendaño-Vázquez, S. E., Dhir, A., Bembich, S., Buratti, E., Proudfoot, N., & Baralle, F. E. (2012). Autoregulation of TDP-43 mRNA levels involves interplay between transcription, splicing, and alternative polyA site selection. *Genes & Development*, *26*, 1679–1684.
14. Baughn, M. W., Melamed, Z., López-Erauskin, J., Beccari, M. S., Ling, K., Zuberi, A., Presa, M., Gonzalo-Gil, E., Maimon, R., Vazquez-Sanchez, S., Chaturvedi, S., Bravo-Hernández, M., Taupin, V., Moore, S., Artates, J. W., Acks, E., Ndayambaje, I. S., Agra De Almeida Quadros, A. R., Jafar-Nejad, P., … Cleveland, D. W. (2023). Mechanism of STMN2 cryptic splice-polyadenylation and its correction for TDP-43 proteinopathies. *Science*, *379*, 1140–1149.
15. Buratti, E., De Conti, L., Stuani, C., Romano, M., Baralle, M., & Baralle, F. (2010). Nuclear factor TDP-43 can affect selected microRNA levels. *FEBS Journal*, *277*, 2268–2281.
16. Kawahara, Y., & Mieda-Sato, A. (2012). TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. *Proceedings of the National Academy of Science USA*, *109*, 3347–3352.
17. Strong, M. J., Volkening, K., Hammond, R., Yang, W., Strong, W., Leystra-Lantz, C., & Shoesmith, C. (2007). TDP43 is a human low molecular weight neurofilament (*h*NFL) mRNA-binding protein. *Molecular and Cellular Neuroscience*, *35*, 320–327.
18. Fiesel, F. C., Voigt, A., Weber, S. S., Van Den Haute, C., Waldenmaier, A., Görner, K., Walter, M., Anderson, M. L., Kern, J. V., Rasse, T. M., Schmidt, T., Springer, W., Kirchner, R., Bonin, M., Neumann, M., Baekelandt, V., Alunni-Fabbroni, M., Schulz, J. B., & Kahle, P. J. (2010). Knockdown of transactive response DNA-binding protein (TDP-43) downregulates histone deacetylase 6. *EMBO Journal*, *29*, 209–221.
19. Yu, Z., Fan, D., Gui, B., Shi, L., Xuan, C., Shan, L., Wang, Q., Shang, Y., & Wang, Y. (2012). Neurodegeneration-associated TDP-43 interacts with fragile X mental retardation protein (FMRP)/Staufen (STAU1) and regulates SIRT1 expression in neuronal cells. *Journal of Biological Chemistry*, *287*, 22560–22572.
20. Neelagandan, N., Gonnella, G., Dang, S., Janiesch, P. C., Miller, K. K., Küchler, K., Marques, R. F., Indenbirken, D., Alawi, M., Grundhoff, A., Kurtz, S., & Duncan, K. E. (2019). TDP-43 enhances translation of specific mRNAs linked to neurodegenerative disease. *Nucleic Acids Research*, *47*, 341–361.
21. Alami, N. H., Smith, R. B., Carrasco, M. A., Williams, L. A., Winborn, C. S., Han, S. S. W., Kiskinis, E., Winborn, B., Freibaum, B. D., Kanagaraj, A., Clare, A. J., Badders, N. M., Bilican, B., Chaum, E., Chandran, S., Shaw, C. E., Eggan, K. C., Maniatis, T., & Taylor, J. P. (2014). Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron*, *81*, 536–543.
22. Colombrita, C., Zennaro, E., Fallini, C., Weber, M., Sommacal, A., Buratti, E., Silani, V., & Ratti, A. (2009). TDP-43 is recruited to stress granules in conditions of oxidative insult. *Journal of Neurochemistry*, *111*, 1051–1061.
23. Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., Bruce, J., Schuck, T., Grossman, M., Clark, C. M., Mccluskey, L. F., Miller, B. L., Masliah, E., Mackenzie, I. R., Feldman, H., Feiden, W., Kretzschmar, H. A., Trojanowski, J. Q., & Lee, V. M.-Y. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*, *314*, 130–133.

24. Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., Mann, D., Tsuchiya, K., Yoshida, M., Hashizume, Y., & Oda, T. (2006). TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochemical and Biophysical Research Communications*, *351*, 602–611.
25. Neumann, M., & Mackenzie, I. R. A. (2019). Review: Neuropathology of non-tau frontotemporal lobar degeneration. *Neuropathology and Applied Neurobiology*, *45*, 19–40.
26. Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J. C., Williams, K. L., Buratti, E., Baralle, F., De Belleroche, J., Mitchell, J. D., Leigh, P. N., Al-Chalabi, A., Miller, C. C., Nicholson, G., & Shaw, C. E. (2008). TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science*, *319*, 1668–1672.
27. Gitcho, M. A., Baloh, R. H., Chakraverty, S., Mayo, K., Norton, J. B., Levitch, D., Hatanpaa, K. J., White, C. L., Bigio, E. H., Caselli, R., Baker, M., Al-Lozi, M. T., Morris, J. C., Pestronk, A., Rademakers, R., Goate, A. M., & Cairns, N. J. (2008). TDP-43 A315T mutation in familial motor neuron disease. *Annals of Neurology*, *63*, 535–538.
28. Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., Mcconkey, B. J., Velde, C. V., Bouchard, J.-P., Lacomblez, L., Pochigaeva, K., Salachas, F., Pradat, P.-F., Camu, W., Meininger, V., Dupre, N., & Rouleau, G. A. (2008). *TARDBP* mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nature Genetics*, *40*, 572–574.
29. Van Deerlin, V. M., Leverenz, J. B., Bekris, L. M., Bird, T. D., Yuan, W., Elman, L. B., Clay, D., Wood, E. M., Chen-Plotkin, A. S., Martinez-Lage, M., Steinbart, E., Mccluskey, L., Grossman, M., Neumann, M., Wu, I.-L., Yang, W.-S., Kalb, R., Galasko, D. R., Montine, T. J., … Yu, C.-E. (2008). TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: A genetic and histopathological analysis. *Lancet Neurology*, *7*, 409–416.
30. Gavin, A.-C., Bösche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J. M., Michon, A.-M., Cruciat, C.-M., Remor, M., Höfert, C., Schelder, M., Brajenovic, M., Ruffner, H., Merino, A., Klein, K., Hudak, M., Dickson, D., … Superti-Furga, G. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature*, *415*, 141–147.
31. Bürckstümmer, T., Bennett, K. L., Preradovic, A., Schütze, G., Hantschel, O., Superti-Furga, G., & Bauch, A. (2006). An efficient tandem affinity purification procedure for interaction proteomics in mammalian cells. *Nature Methods*, *3*, 1013–1019.
32. Gloeckner, C. J., Boldt, K., Schumacher, A., Roepman, R., & Ueffing, M. (2007). A novel tandem affinity purification strategy for the efficient isolation and characterisation of native protein complexes. *Proteomics*, *7*, 4228–4234.
33. Boldt, K., Van Reeuwijk, J., Lu, Q., Koutroumpas, K., Nguyen, T.-M. T., Texier, Y., Van Beersum, S. E. C., Horn, N., Willer, J. R., Mans, D. A., Dougherty, G., Lamers, I. J. C., Coene, K. L. M., Arts, H. H., Betts, M. J., Beyer, T., Bolat, E., Gloeckner, C. J., Haidari, K., … Williamson, K. (2016). An organelle-specific protein landscape identifies novel diseases and molecular mechanisms. *Nature Communications*, *7*, 11491.
34. Ino, Y., Yamaoka, Y., Tanaka, K., Miyakawa, K., Nishi, M., Hatayama, Y., Kimura, H., Kimura, Y., & Ryo, A. (2023). Integrated tandem affinity protein purification using the polyhistidine plus extra 4 amino acids (HiP4) tag system. *Proteomics*, *23*, e2200334.
35. Brymora, A., Valova, V. A., & Robinson, P. J. (2004). Protein-protein interactions identified by pull-down experiments and mass spectrometry. *Current Protocols in Cell Biology*, Chapter 17, Unit 17.5.
36. Pourhaghighi, R., Ash, P. E. A., Phanse, S., Goebels, F., Hu, L. Z. M., Chen, S., Zhang, Y., Wierbowski, S. D., Boudeau, S., Moutaoufik, M. T., Malty, R. H., Malolepsza, E., Tsafou, K., Nathan, A., Cromar, G., Guo, H., Abdullatif, A. A., Apicco, D. J., Becker, L. A., … Emili, A. (2020). BraInMap elucidates the macromolecular connectivity landscape of mammalian brain. *Cell Systems*, *10*, 333–350.e14.
37. Selbach, M., & Mann, M. (2006). Protein interaction screening by quantitative immunoprecipitation combined with knockdown (QUICK). *Nature Methods*, *3*, 981–983.
38. Meixner, A., Boldt, K., Van Troys, M., Askenazi, M., Gloeckner, C. J., Bauer, M., Marto, J. A., Ampe, C., Kinkl, N., & Ueffing, M. (2011). A QUICK Screen for Lrrk2 Interaction Partners—Leucine-rich Repeat Kinase 2 is Involved in Actin Cytoskeleton Dynamics. *Molecular & Cellular Proteomics*, *10*, M110.001172.
39. Riemenschneider, H., Guo, Q., Bader, J., Frottin, F., Farny, D., Kleinberger, G., Haass, C., Mann, M., Hartl, F. U., Baumeister, W., Hipp, M. S., Meissner, F., Fernández-Busnadiego, R., & Edbauer, D. (2022). Gel-like inclusions of C-terminal fragments of TDP-43 sequester stalled proteasomes in neurons. *EMBO Reports*, *23*, e53890.
40. Kawaguchi, T., Rollins, M. G., Moinpour, M., Morera, A. A., Ebmeier, C. C., Old, W. M., & Schwartz, J. C. (2020). Changes to the TDP-43 and FUS interactomes induced by DNA Damage. *Journal of Proteome Research*, *19*, 360–370.
41. Feneberg, E., Gordon, D., Thompson, A. G., Finelli, M. J., Dafinca, R., Candalija, A., Charles, P. D., Mäger, I., Wood, M. J., Fischer, R., Kessler, B. M., Gray, E., Turner, M. R., & Talbot, K. (2020). An ALS-linked mutation in TDP-43 disrupts normal protein interactions in the motor neuron response to oxidative stress. *Neurobiology of Disease*, *144*, 105050.
42. Freibaum, B. D., Chitta, R. K., High, A. A., & Taylor, J. P. (2010). Global analysis of TDP-43 interacting proteins reveals strong association with RNA splicing and translation machinery. *Journal of Proteome Research*, *9*, 1104–1120.
43. Ling, S.-C., Albuquerque, C. P., Han, J. S., Lagier-Tourenne, C., Tokunaga, S., Zhou, H., & Cleveland, D. W. (2010). ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. *Proceedings National Academy of Science USA*, *107*, 13318–13323.
44. Ratushny, V., & Golemis, E. A. (2008). Resolving the network of cell signaling pathways using the evolving yeast two-hybrid system. *Biotechniques*, *44*, 655–662.
45. Haenig, C., Atias, N., Taylor, A. K., Mazza, A., Schaefer, M. H., Russ, J., Riechers, S.-P., Jain, S., Coughlin, M., Fontaine, J.-F., Freibaum, B. D., Brusendorf, L., Zenkner, M., Porras, P., Stroedicke, M., Schnoegl, S., Arnsburg, K., Boeddrich, A., Pigazzini, L., … Wanker, E. E. (2020). Interactome mapping provides a network of neurodegenerative disease proteins and uncovers widespread protein aggregation in affected brains. *Cell Reports*, *32*, 108050.
46. Hans, F., Fiesel, F. C., Strong, J. C., Jäckel, S., Rasse, T. M., Geisler, S., Springer, W., Schulz, J. B., Voigt, A., & Kahle, P. J. (2014). UBE2E ubiquitin-conjugating enzymes and ubiquitin isopeptidase Y regulate TDP-43 protein ubiquitination. *Journal of Biological Chemistry*, *289*, 19164–19179.
47. Pfeiffer, C. T., Paulo, J. A., Gygi, S. P., & Rockman, H. A. (2022). Proximity labeling for investigating protein-protein interactions. *Methods in Cell Biology*, *169*, 237–266.
48. Chou, C.-C., Zhang, Y., Umoh, M. E., Vaughan, S. W., Lorenzini, I., Liu, F., Sayegh, M., Donlin-Asp, P. G., Chen, Y. H., Duong, D. M., Seyfried, N. T., Powers, M. A., Kukar, T., Hales, C. M., Gearing, M., Cairns, N. J., Boylan, K. B., Dickson, D. W., Rademakers, R., … Rossoll, W. (2018). TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. *Nature Neuroscience*, *21*, 228–239.
49. Saini, H. K., Griffiths-Jones, S., & Enright, A. J. (2007). Genomic analysis of human microRNA transcripts. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 17719–17724.
50. Shukla, G. C., Singh, J., & Barik, S. (2011). MicroRNAs: Processing, maturation, target recognition and regulatory functions. *Molecular and Cellular Pharmacology*, *3*, 83–92.

51. Kim, K. Y., Lee, H.-W., Shim, Y.-M., Mook-Jung, I., Jeon, G. S., & Sung, J.-J. (2015). A phosphomimetic mutant TDP-43 (S409/410E) induces Drosha instability and cytotoxicity in Neuro 2A cells. *Biochemical and Biophysical Research Communications*, *464*, 236–243.
52. Hawley, Z. C. E., Campos-Melo, D., & Strong, M. J. (2020). Evidence of a negative feedback network between TDP-43 and miRNAs dependent on TDP-43 nuclear localization. *Journal of Molecular Biology*, *432*, 166695.
53. Di Carlo, V., Grossi, E., Laneve, P., Morlando, M., Dini Modigliani, S., Ballarino, M., Bozzoni, I., & Caffarelli, E. (2013). TDP-43 regulates the microprocessor complex activity during in vitro neuronal differentiation. *Molecular Neurobiology*, *48*, 952–963.
54. Kocerha, J., Kouri, N., Baker, M., Finch, N., Dejesus-Hernandez, M., Gonzalez, J., Chidamparam, K., Josephs, K. A., Boeve, B. F., Graff-Radford, N. R., Crook, J., Dickson, D. W., & Rademakers, R. (2011). Altered microRNA expression in frontotemporal lobar degeneration with TDP-43 pathology caused by progranulin mutations. *BMC Genomics*, *12*, 527.
55. Mohagheghi, F., Prudencio, M., Stuani, C., Cook, C., Jansen-West, K., Dickson, D. W., Petrucelli, L., & Buratti, E. (2016). TDP-43 functions within a network of hnRNP proteins to inhibit the production of a truncated human SORT1 receptor. *Human Molecular Genetics*, *25*, 534–545.
56. Buratti, E., Brindisi, A., Giombi, M., Tisminetzky, S., Ayala, Y. M., & Baralle, F. E. (2005). TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through Its C-terminal Tail. *Journal of Biological Chemistry*, *280*, 37572–37584.
57. Koike, Y., Pickles, S., Estades Ayuso, V., Jansen-West, K., Qi, Y. A., Li, Z., Daughrity, L. M., Yue, M., Zhang, Y.-J., Cook, C. N., Dickson, D. W., Ward, M., Petrucelli, L., & Prudencio, M. (2023). TDP-43 and other hnRNPs regulate cryptic exon inclusion of a key ALS/FTD risk gene, UNC13A. *PLoS Biology*, *21*, e3002028.
58. Bishof, I., Dammer, E. B., Duong, D. M., Kundinger, S. R., Gearing, M., Lah, J. J., Levey, A. I., & Seyfried, N. T. (2018). RNA-binding proteins with basic-acidic dipeptide (BAD) domains self-assemble and aggregate in Alzheimer's disease. *Journal of Biological Chemistry*, *293*, 11047–11066.
59. Wilkinson, M. E., Charenton, C., & Nagai, K. (2020). RNA splicing by the spliceosome. *Annual Review of Biochemistry*, *89*, 359–388.
60. Bergfort, A., Preußner, M., Kuropka, B., Ilik, I. A., Hilal, T., Weber, G., Freund, C., Aktas, T., Heyd, F., & Wahl, M. C. (2022). A multi-factor trafficking site on the spliceosome remodeling enzyme BRR2 recruits C9ORF78 to regulate alternative splicing. *Nature Communications*, *13*, 1132.
61. Mund, M., Neu, A., Ullmann, J., Neu, U., & Sprangers, R. (2011). Structure of the LSm657 Complex: An Assembly Intermediate of the LSm1–7 and LSm2–8 Rings. *Journal of Molecular Biology*, *414*, 165–176.
62. Bertram, K., Agafonov, D. E., Dybkov, O., Haselbach, D., Leelaram, M. N., Will, C. L., Urlaub, H., Kastner, B., Lührmann, R., & Stark, H. (2017). Cryo-EM structure of a pre-catalytic human spliceosome primed for activation. *Cell*, *170*, 701–713.e11.
63. Bouveret, E. (2000). A Sm-like protein complex that participates in mRNA degradation. *EMBO Journal*, *19*, 1661–1671.
64. Ingelfinger, D., Arndt-Jovin, D. J., Lührmann, R., & Achsel, T. (2002). The human LSm1-7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrn1 in distinct cytoplasmic foci. *RNA*, *8*, 1489–1501.
65. Tharun, S., He, W., Mayes, A. E., Lennertz, P., Beggs, J. D., & Parker, R. (2000). Yeast Sm-like proteins function in mRNA decapping and decay. *Nature*, *404*, 515–518.
66. Vindry, C., Weil, D., & Standart, N. (2019). Pat1 RNA-binding proteins: Multitasking shuttling proteins. *Wiley Interdiscip Rev RNA*, *10*, e1557.
67. Sachdev, R., Hondele, M., Linsenmeier, M., Vallotton, P., Mugler, C. F., Arosio, P., & Weis, K. (2019). Pat1 promotes processing body assembly by enhancing the phase separation of the DEAD-box ATPase Dhh1 and RNA. *Elife*, *8*, e41415.
68. Lobel, J. H., & Gross, J. D. (2020). Pdc2/Pat1 increases the range of decay factors and RNA bound by the Lsm1-7 complex. *RNA*, *26*, 1380–1388.
69. Ayala, Y. M., De Conti, L., Avendaño-Vázquez, S. E., Dhir, A., Romano, M., D'ambrogio, A., Tollervey, J., Ule, J., Baralle, M., Buratti, E., & Baralle, F. E. (2011). TDP-43 regulates its mRNA levels through a negative feedback loop. *EMBO Journal*, *30*, 277–288.
70. Koyama, A., Sugai, A., Kato, T., Ishihara, T., Shiga, A., Toyoshima, Y., Koyama, M., Konno, T., Hirokawa, S., Yokoseki, A., Nishizawa, M., Kakita, A., Takahashi, H., & Onodera, O. (2016). Increased cytoplasmic *TARDBP* mRNA in affected spinal motor neurons in ALS caused by abnormal autoregulation of TDP-43. *Nucleic Acids Research*, *44*, 5820–5836.
71. Igaz, L. M., Kwong, L. K., Lee, E. B., Chen-Plotkin, A., Swanson, E., Unger, T., Malunda, J., Xu, Y., Winton, M. J., Trojanowski, J. Q., & Lee, V. M.-Y. (2011). Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. *Journal of Clinical Investigation*, *121*, 726–738.
72. Gitcho, M. A., Bigio, E. H., Mishra, M., Johnson, N., Weintraub, S., Mesulam, M., Rademakers, R., Chakraverty, S., Cruchaga, C., Morris, J. C., Goate, A. M., & Cairns, N. J. (2009). TARDBP 3′-UTR variant in autopsy-confirmed frontotemporal lobar degeneration with TDP-43 proteinopathy. *Acta Neuropathologica*, *118*, 633–645.
73. Koehler, L. C., Grese, Z. R., Bastos, A. C. S., Mamede, L. D., Heyduk, T., & Ayala, Y. M. (2022). TDP-43 oligomerization and phase separation properties are necessary for autoregulation. *Frontiers in Neuroscience*, *16*, 818655.
74. Bembich, S., Herzog, J. S., De Conti, L., Stuani, C., Avendaño-Vázquez, S. E., Buratti, E., Baralle, M., & Baralle, F. E. (2014). Predominance of spliceosomal complex formation over polyadenylation site selection in TDP-43 autoregulation. *Nucleic Acids Research*, *42*, 3362–3371.
75. Torres, P., Ramírez-Núñez, O., Romero-Guevara, R., Barés, G., Granado-Serrano, A. B., Ayala, V., Boada, J., Fontdevila, L., Povedano, M., Sanchís, D., Pamplona, R., Ferrer, I., & Portero-Otín, M. (2018). Cryptic exon splicing function of TARDBP interacts with autophagy in nervous tissue. *Autophagy*, *14*, 1398–1403.
76. Sephton, C. F., Cenik, C., Kucukural, A., Dammer, E. B., Cenik, B., Han, Y., Dewey, C. M., Roth, F. P., Herz, J., Peng, J., Moore, M. J., & Yu, G. (2011). Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. *Journal of Biological Chemistry*, *286*, 1204–1215.
77. Colombrita, C., Onesto, E., Megiorni, F., Pizzuti, A., Baralle, F. E., Buratti, E., Silani, V., & Ratti, A. (2012). TDP-43 and FUS RNA-binding proteins bind distinct sets of cytoplasmic messenger RNAs and differently regulate their post-transcriptional fate in motoneuron-like cells. *Journal of Biological Chemistry*, *287*, 15635–15647.
78. Caccamo, A., Majumder, S., Deng, J. J., Bai, Y., Thornton, F. B., & Oddo, S. (2009). Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability. *Journal of Biological Chemistry*, *284*, 27416–27424.
79. Sidibé, H., Khalfallah, Y., Xiao, S., Gómez, N. B., Fakim, H., Tank, E. M. H., Di Tomasso, G., Bareke, E., Aulas, A., Mckeever, P. M., Melamed, Z., Destroimaisons, L., Deshaies, J.-E., Zinman, L., Parker, J. A., Legault, P., Tétreault, M., Barmada, S. J., Robertson, J., … Vande Velde, C. (2021). TDP-43 stabilizes *G3BP1* mRNA: Relevance to amyotrophic lateral sclerosis/frontotemporal dementia. *Brain*, *144*, 3461–3476.
80. McDonald, K. K., Aulas, A., Destroismaisons, L., Pickles, S., Beleac, E., Camu, W., Rouleau, G. A., & Vande Velde, C. (2011). TAR DNA-binding protein 43 (TDP-43) regulates stress granule dynamics via

differential regulation of G3BP and TIA-1. *Human Molecular Genetics*, *20*, 1400–1410.
81. Kim, S. H., Shanware, N. P., Bowler, M. J., & Tibbetts, R. S. (2010). Amyotrophic Lateral Sclerosis-associated Proteins TDP-43 and FUS/TLS Function in a Common Biochemical Complex to Co-regulate HDAC6 mRNA. *Journal of Biological Chemistry*, *285*, 34097–34105.
82. Müller-McNicoll, M., Botti, V., De Jesus Domingues, A. M., Brandl, H., Schwich, O. D., Steiner, M. C., Curk, T., Poser, I., Zarnack, K., & Neugebauer, K. M. (2016). SR proteins are NXF1 adaptors that link alternative RNA processing to mRNA export. *Genes & Development*, *30*, 553–566.
83. Fallini, C., Bassell, G. J., & Rossoll, W. (2012). The ALS disease protein TDP-43 is actively transported in motor neuron axons and regulates axon outgrowth. *Human Molecular Genetics*, *21*, 3703–3718.
84. Chu, J.-F., Majumder, P., Chatterjee, B., Huang, S.-L., & Shen, C.-K. J. (2019). TDP-43 Regulates Coupled Dendritic mRNA Transport-Translation Processes in Co-operation with FMRP and Staufen1. *Cell Reports*, *29*, 3118–3133.e6.
85. Vishal, S. S., Wijegunawardana, D., Salaikumaran, M. R., & Gopal, P. P. (2022). Sequence Determinants of TDP-43 Ribonucleoprotein Condensate Formation and Axonal Transport in Neurons. *Frontiers in Cell and Developmental Biology*, *10*, 876893.
86. Gopal, P. P., Nirschl, J. J., Klinman, E., & Holzbaur, E. L. F. (2017). Amyotrophic lateral sclerosis-linked mutations increase the viscosity of liquid-like TDP-43 RNP granules in neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E2466–E2475.
87. Anderson, P., & Kedersha, N. (2009). RNA granules: Post-transcriptional and epigenetic modulators of gene expression. *Nature Reviews Molecular Cell Biology*, *10*, 430–436.
88. Jain, S., Wheeler, J. R., Walters, R. W., Agrawal, A., Barsic, A., & Parker, R. (2016). ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell*, *164*, 487–498.
89. Kedersha, N., Chen, S., Gilks, N., Li, W., Miller, I. J., Stahl, J., & Anderson, P. (2002). Evidence that ternary complex (eIF2-GTP-tRNA iMet)–deficient preinitiation complexes are core constituents of mammalian stress granules. *Molecular Biology of the Cell*, *13*, 195–210.
90. Mateju, D., Eichenberger, B., Voigt, F., Eglinger, J., Roth, G., & Chao, J. A. (2020). Single-molecule imaging reveals translation of mRNAs localized to stress granules. *Cell*, *183*, 1801–1812.e13.
91. Putnam, A., Thomas, L., & Seydoux, G. (2023). RNA granules: Functional compartments or incidental condensates? *Genes & Development*, *37*, 354–376.
92. Liu-Yesucevitz, L., Bilgutay, A., Zhang, Y.-J., Vanderwyde, T., Citro, A., Mehta, T., Zaarur, N., Mckee, A., Bowser, R., Sherman, M., Petrucelli, L., & Wolozin, B. (2010). Tar DNA binding protein-43 (TDP-43) associates with stress granules: Analysis of cultured cells and pathological brain tissue. *PLoS ONE*, *5*, e13250.
93. Aulas, A., Stabile, S., & Vande Velde, C. (2012). Endogenous TDP-43, but not FUS, contributes to stress granule assembly via G3BP. *Molecular Neurodegeneration*, *7*, 54.
94. Zhang, P., Fan, B., Yang, P., Temirov, J., Messing, J., Kim, H. J., & Taylor, J. P. (2019). Chronic optogenetic induction of stress granules is cytotoxic and reveals the evolution of ALS-FTD pathology. *Elife*, *8*, e39578.
95. Chen, Y., & Cohen, T. J. (2019). Aggregation of the nucleic acid–binding protein TDP-43 occurs via distinct routes that are coordinated with stress granule formation. *Journal of Biological Chemistry*, *294*, 3696–3706.
96. Hans, F., Glasebach, H., & Kahle, P. J. (2020). Multiple distinct pathways lead to hyperubiquitylated insoluble TDP-43 protein independent of its translocation into stress granules. *Journal of Biological Chemistry*, *295*, 673–689.
97. Charif, S. E., Luchelli, L., Vila, A., Blaustein, M., & Igaz, L. M. (2020). Cytoplasmic expression of the ALS/FTD-related protein TDP-43 decreases global translation both in vitro and in vivo. *Frontiers in Cellular Neuroscience*, *14*, 594561.
98. Russo, A., Scardigli, R., La Regina, F., Murray, M. E., Romano, N., Dickson, D. W., Wolozin, B., Cattaneo, A., & Ceci, M. (2017). Increased cytoplasmic TDP-43 reduces global protein synthesis by interacting with RACK1 on polyribosomes. *Human Molecular Genetics*, *26*, 1407–1418.
99. MacNair, L., Xiao, S., Miletic, D., Ghani, M., Julien, J.-P., Keith, J., Zinman, L., Rogaeva, E., & Robertson, J. (2016). MTHFSD and DDX58 are novel RNA-binding proteins abnormally regulated in amyotrophic lateral sclerosis. *Brain*, *139*, 86–100.
100. Altman, T., Ionescu, A., Ibraheem, A., Priesmann, D., Gradus-Pery, T., Farberov, L., Alexandra, G., Shelestovich, N., Dafinca, R., Shomron, N., Rage, F., Talbot, K., Ward, M. E., Dori, A., Krüger, M., & Perlson, E. (2021). Axonal TDP-43 condensates drive neuromuscular junction disruption through inhibition of local synthesis of nuclear encoded mitochondrial proteins. *Nature Communications*, *12*, 6914.
101. Wong, C.-E., Jin, L.-W., Chu, Y.-P., Wei, W.-Y., Ho, P.-C., & Tsai, K.-J. (2021). TDP-43 proteinopathy impairs mRNP granule mediated postsynaptic translation and mRNA metabolism. *Theranostics*, *11*, 330–345.
102. Fiesel, F. C., Weber, S. S., Supper, J., Zell, A., & Kahle, P. J. (2012). TDP-43 regulates global translational yield by splicing of exon junction complex component SKAR. *Nucleic Acids Research*, *40*, 2668–2682.
103. Nagano, S., Jinno, J., Abdelhamid, R. F., Jin, Y., Shibata, M., Watanabe, S., Hirokawa, S., Nishizawa, M., Sakimura, K., Onodera, O., Okada, H., Okada, T., Saito, Y., Takahashi-Fujigasaki, J., Murayama, S., Wakatsuki, S., Mochizuki, H., & Araki, T. (2020). TDP-43 transports ribosomal protein mRNA to regulate axonal local translation in neuronal axons. *Acta Neuropathologica*, *140*, 695–713.
104. Bjork, R. T., Mortimore, N. P., Loganathan, S., & Zarnescu, D. C. (2022). Dysregulation of translation in TDP-43 proteinopathies: Deficits in the RNA supply chain and local protein production. *Frontiers in Neuroscience*, *16*, 840357.
105. Guo, F., Jiao, F., Song, Z., Li, S., Liu, B., Yang, H., Zhou, Q., & Li, Z. (2015). Regulation of MALAT1 expression by TDP43 controls the migration and invasion of non-small cell lung cancer cells in vitro. *Biochemical and Biophysical Research Communications*, *465*, 293–298.
106. Nguyen, T. M., Kabotyanski, E. B., Reineke, L. C., Shao, J., Xiong, F., Lee, J.-H., Dubrulle, J., Johnson, H., Stossi, F., Tsoi, P. S., Choi, K.-J., Ellis, A. G., Zhao, N., Cao, J., Adewunmi, O., Ferreon, J. C., Ferreon, A. C. M., Neilson, J. R., Mancini, M. A., … Rosen, J. M. (2020). The SINEB1 element in the long non-coding RNA *Malat1* is necessary for TDP-43 proteostasis. *Nucleic Acids Research*, *48*, 2621–2642.
107. Nishimoto, Y., Nakagawa, S., Hirose, T., Okano, H., Takao, M., Shibata, S., Suyama, S., Kuwako, K.-I., Imai, T., Murayama, S., Suzuki, N., & Okano, H. (2013). The long non-coding RNA nuclear-enriched abundant transcript 1_2 induces paraspeckle formation in the motor neuron during the early phase of amyotrophic lateral sclerosis. *Molecular Brain*, *6*, 31.
108. Modic, M., Grosch, M., Rot, G., Schirge, S., Lepko, T., Yamazaki, T., Lee, F. C. Y., Rusha, E., Shaposhnikov, D., Palo, M., Merl-Pham, J., Cacchiarelli, D., Rogelj, B., Hauck, S. M., Von Mering, C., Meissner, A., Lickert, H., Hirose, T., Ule, J., & Drukker, M. (2019). Cross-Regulation between TDP-43 and Paraspeckles Promotes Pluripotency-Differentiation Transition. *Molecular Cell*, *74*, 951–965.e13.
109. Wang, C., Duan, Y., Duan, G., Wang, Q., Zhang, K., Deng, X., Qian, B., Gu, J., Ma, Z., Zhang, S., Guo, L., Liu, C., & Fang, Y. (2020). Stress induces dynamic, cytotoxicity-antagonizing TDP-43 nuclear bodies via paraspeckle LncRNA NEAT1-mediated liquid-liquid phase separation. *Molecular Cell*, *79*, 443–458.e7.

110. Shelkovnikova, T. A., Kukharsky, M. S., An, H., Dimasi, P., Alexeeva, S., Shabir, O., Heath, P. R., & Buchman, V. L. (2018). Protective paraspeckle hyper-assembly downstream of TDP-43 loss of function in amyotrophic lateral sclerosis. *Molecular Neurodegeneration*, *13*, 30.
111. Suzuki, H., Shibagaki, Y., Hattori, S., & Matsuoka, M. (2019). C9-ALS/FTD-linked proline–arginine dipeptide repeat protein associates with paraspeckle components and increases paraspeckle formation. *Cell Death & Disease*, *10*, 746.
112. Matsukawa, K., Kukharsky, M. S., Park, S.-K., Park, S., Watanabe, N., Iwatsubo, T., Hashimoto, T., Liebman, S. W., & Shelkovnikova, T. A. (2021). Long non-coding RNA NEAT1_1 ameliorates TDP-43 toxicity in in vivo models of TDP-43 proteinopathy. *RNA Biology*, *18*, 1546–1554.
113. Sekar, D., Tusubira, D., & Ross, K. (2022). TDP-43 and NEAT long noncoding RNA: Roles in neurodegenerative disease. *Frontiers in Cellular Neuroscience*, *16*, 954912.
114. Liu, X., Li, D., Zhang, W., Guo, M., & Zhan, Q. (2012). Long non-coding RNA *gadd7* interacts with TDP-43 and regulates *Cdk6* mRNA decay. *EMBO Journal*, *31*, 4415–4427.
115. Keihani, S., Kluever, V., Mandad, S., Bansal, V., Rahman, R., Fritsch, E., Gomes, L. C., Gärtner, A., Kügler, S., Urlaub, H., Wren, J. D., Bonn, S., Rizzoli, S. O., & Fornasiero, E. F. (2019). The long noncoding RNA *neuroLNC* regulates presynaptic activity by interacting with the neurodegeneration-associated protein TDP-43. *Science Advances*, *5*, eaay2670.
116. Balas, M. M., Porman, A. M., Hansen, K. C., & Johnson, A. M. (2018). SILAC-MS profiling of reconstituted human chromatin platforms for the study of transcription and RNA regulation. *Journal of Proteome Research*, *17*, 3475–3484.
117. Pandya-Jones, A., Markaki, Y., Serizay, J., Chitiashvili, T., Mancia Leon, W. R., Damianov, A., Chronis, C., Papp, B., Chen, C.-K., Mckee, R., Wang, X.-J., Chau, A., Sabri, S., Leonhardt, H., Zheng, S., Guttman, M., Black, D. L., & Plath, K. (2020). A protein assembly mediates Xist localization and gene silencing. *Nature*, *587*, 145–151.
118. Lu, Z., Guo, J. K., Wei, Y., Dou, D. R., Zarnegar, B., Ma, Q., Li, R., Zhao, Y., Liu, F., Choudhry, H., Khavari, P. A., & Chang, H. Y. (2020). Structural modularity of the XIST ribonucleoprotein complex. *Nature Communications*, *11*, 6163.
119. Ayala, Y. M., Zago, P., D'ambrogio, A., Xu, Y.-F., Petrucelli, L., Buratti, E., & Baralle, F. E. (2008). Structural determinants of the cellular localization and shuttling of TDP-43. *Journal of Cell Science*, *121*, 3778–3785.
120. Pinarbasi, E. S., Cagatay, T., Fung, H. Y. J., Li, Y. C., Chook, Y. M., & Thomas, P. J. (2018). Active nuclear import and passive nuclear export are the primary determinants of TDP-43 localization. *Scientific Reports*, *8*, 7083.
121. Doll, S. G., Meshkin, H., Bryer, A. J., Li, F., Ko, Y.-H., Lokareddy, R. K., Gillilan, R. E., Gupta, K., Perilla, J. R., & Cingolani, G. (2022). Recognition of the TDP-43 nuclear localization signal by importin $\alpha 1/\beta$. *Cell Reports*, *39*, 111007.
122. Garcia Morato, J., Hans, F., Von Zweydorf, F., Feederle, R., Elsässer, S. J., Skodras, A. A., Gloeckner, C. J., Buratti, E., Neumann, M., & Kahle, P. J. (2022). Sirtuin-1 sensitive lysine-136 acetylation drives phase separation and pathological aggregation of TDP-43. *Nature Communications*, *13*, 1223.
123. Hans, F., Eckert, M., Von Zweydorf, F., Gloeckner, C. J., & Kahle, P. J. (2018). Identification and characterization of ubiquitinylation sites in TAR DNA-binding protein of 43 kDa (TDP-43). *Journal of Biological Chemistry*, *293*, 16083–16099.
124. Winton, M. J., Igaz, L. M., Wong, M. M., Kwong, L. K., Trojanowski, J. Q., & Lee, V. M.-Y. (2008). Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. *Journal of Biological Chemistry*, *283*, 13302–13309.
125. Gasset-Rosa, F., Lu, S., Yu, H., Chen, C., Melamed, Z., Guo, L., Shorter, J., Da Cruz, S., & Cleveland, D. W. (2019). Cytoplasmic TDP-43 demixing independent of stress granules drives inhibition of nuclear import, loss of nuclear TDP-43, and cell death. *Neuron*, *102*, 339–357.e7.
126. Khalil, B., Chhangani, D., Wren, M. C., Smith, C. L., Lee, J. H., Li, X., Puttinger, C., Tsai, C.-W., Fortin, G., Morderer, D., Gao, J., Liu, F., Lim, C. K., Chen, J., Chou, C.-C., Croft, C. L., Gleixner, A. M., Donnelly, C. J., Golde, T. E., … Rossoll, W. (2022). Nuclear import receptors are recruited by FG-nucleoporins to rescue hallmarks of TDP-43 proteinopathy. *Molecular Neurodegeneration*, *17*, 80.
127. Cohen, T. J., Hwang, A. W., Unger, T., Trojanowski, J. Q., & Lee, V. M. Y. (2012). Redox signalling directly regulates TDP-43 via cysteine oxidation and disulphide cross-linking. *EMBO Journal*, *31*, 1241–1252.
128. Pirie, E., Oh, C., Zhang, X., Han, X., Cieplak, P., Scott, H. R., Deal, A. K., Ghatak, S., Martinez, F. J., Yeo, G. W., Yates, J. R., Nakamura, T., & Lipton, S. A. (2021). S-nitrosylated TDP-43 triggers aggregation, cell-to-cell spread, and neurotoxicity in hiPSCs and in vivo models of ALS/FTD. *Proceedings of the National Academy of Sciences of the United States of America*, *118*, e2021368118.
129. Inukai, Y., Nonaka, T., Arai, T., Yoshida, M., Hashizume, Y., Beach, T. G., Buratti, E., Baralle, F. E., Akiyama, H., Hisanaga, S.-I., & Hasegawa, M. (2008). Abnormal phosphorylation of Ser409/410 of TDP-43 in FTLD-U and ALS. *FEBS Letters*, *582*, 2899–2904.
130. Neumann, M., Kwong, L. K., Lee, E. B., Kremmer, E., Flatley, A., Xu, Y., Forman, M. S., Troost, D., Kretzschmar, H. A., Trojanowski, J. Q., & Lee, V. M.-Y. (2009). Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. *Acta Neuropathologica*, *117*, 137–149.
131. Hasegawa, M., Arai, T., Nonaka, T., Kametani, F., Yoshida, M., Hashizume, Y., Beach, T. G., Buratti, E., Baralle, F., Morita, M., Nakano, I., Oda, T., Tsuchiya, K., & Akiyama, H. (2008). Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Annals of Neurology*, *64*, 60–70.
132. Neumann, M., Frick, P., Paron, F., Kosten, J., Buratti, E., & Mackenzie, I. R. (2021). Correction to: Antibody against TDP-43 phosphorylated at serine 369 suggests conformational differences of TDP-43 aggregates among FTLD-TDP subtypes. *Acta Neuropathologica*, *141*, 137.
133. Kametani, F., Obi, T., Shishido, T., Akatsu, H., Murayama, S., Saito, Y., Yoshida, M., & Hasegawa, M. (2016). Mass spectrometric analysis of accumulated TDP-43 in amyotrophic lateral sclerosis brains. *Scientific Reports*, *6*, 23281.
134. Okochi, M., Walter, J., Koyama, A., Nakajo, S., Baba, M., Iwatsubo, T., Meijer, L., Kahle, P. J., & Haass, C. (2000). Constitutive Phosphorylation of the Parkinson's Disease Associated α-Synuclein. *Journal of Biological Chemistry*, *275*, 390–397.
135. Singh, T. J., Grundke-Iqbal, I., & Iqbal, K. (1995). Phosphorylation of τ protein by Casein Kinase-1 converts it to an abnormal Alzheimer-like state. *Journal of Neurochemistry*, *64*, 1420–1423.
136. Choksi, D. K., Roy, B., Chatterjee, S., Yusuff, T., Bakhoum, M. F., Sengupta, U., Ambegaokar, S., Kayed, R., & Jackson, G. R. (2014). TDP-43 Phosphorylation by casein kinase Iε promotes oligomerization and enhances toxicity in vivo. *Human Molecular Genetics*, *23*, 1025–1035.
137. Nonaka, T., Suzuki, G., Tanaka, Y., Kametani, F., Hirai, S., Okado, H., Miyashita, T., Saitoe, M., Akiyama, H., Masai, H., & Hasegawa, M. (2016). Phosphorylation of TAR DNA-binding protein of 43 kDa (TDP-43) by Truncated Casein Kinase 1δ triggers mislocalization and accumulation of TDP-43. *Journal of Biological Chemistry*, *291*, 5473–5483.
138. Hicks, D. A., Cross, L. L., Williamson, R., & Rattray, M. (2020). Endoplasmic reticulum stress signalling induces Casein Kinase 1-

dependent formation of cytosolic TDP-43 inclusions in motor neuron-like cells. *Neurochemical Research*, *45*, 1354–1364.

139. Martínez-González, L., Rodríguez-Cueto, C., Cabezudo, D., Bartolomé, F., Andrés-Benito, P., Ferrer, I., Gil, C., Martín-Requero, Á., Fernández-Ruiz, J., Martínez, A., & de Lago, E. (2020). Motor neuron preservation and decrease of in vivo TDP-43 phosphorylation by protein CK-1delta kinase inhibitor treatment. *Scientific Reports*, *10*, 4449.
140. Salado, I. G., Redondo, M., Bello, M. L., Perez, C., Liachko, N. F., Kraemer, B. C., Miguel, L., Lecourtois, M., Gil, C., Martinez, A., & Perez, D. I. (2014). Protein kinase CK-1 inhibitors as new potential drugs for amyotrophic lateral sclerosis. *Journal of Medicinal Chemistry*, *57*, 2755–2772.
141. Liachko, N. F., McMillan, P. J., Guthrie, C. R., Bird, T. D., Leverenz, J. B., & Kraemer, B. C. (2013). CDC7 inhibition blocks pathological TDP-43 phosphorylation and neurodegeneration. *Annals of Neurology*, *74*, 39–52.
142. Rojas-Prats, E., Martinez-Gonzalez, L., Gonzalo-Consuegra, C., Liachko, N. F., Perez, C., Ramírez, D., Kraemer, B. C., Martin-Requero, Á., Perez, D. I., Gil, C., De Lago, E., & Martinez, A. (2021). Targeting nuclear protein TDP-43 by cell division cycle kinase 7 inhibitors: A new therapeutic approach for amyotrophic lateral sclerosis. *European Journal of Medicinal Chemistry*, *210*, 112968.
143. Vaca, G., Martinez-Gonzalez, L., Fernandez, A., Rojas-Prats, E., Porras, G., Cuevas, E. P., Gil, C., Martinez, A., & Martin-Requero, Á. (2021). Therapeutic potential of novel Cell Division Cycle Kinase 7 inhibitors on TDP-43-related pathogenesis such as Frontotemporal Lobar Degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). *Journal of Neurochemistry*, *156*, 379–390.
144. Liachko, N. F., Mcmillan, P. J., Strovas, T. J., Loomis, E., Greenup, L., Murrell, J. R., Ghetti, B., Raskind, M. A., Montine, T. J., Bird, T. D., Leverenz, J. B., & Kraemer, B. C. (2014). The tau tubulin kinases TTBK1/2 promote accumulation of pathological TDP-43. *PLoS Genetics*, *10*, e1004803.
145. Sato, S., Cerny, R. L., Buescher, J. L., & Ikezu, T. (2006). Tau-tubulin kinase 1 (TTBK1), a neuron-specific tau kinase candidate, is involved in tau phosphorylation and aggregation. *Journal of Neurochemistry*, *98*, 1573–1584.
146. Tian, Y., Wang, Y., Jablonski, A. M., Hu, Y., Sugam, J. A., Koglin, M., Stachel, S. J., Zhou, H., Uslaner, J. M., & Parmentier-Batteur, S. (2021). Tau-tubulin kinase 1 phosphorylates TDP-43 at disease-relevant sites and exacerbates TDP-43 pathology. *Neurobiology of Disease*, *161*, 105548.
147. Nozal, V., Martínez-González, L., Gomez-Almeria, M., Gonzalo-Consuegra, C., Santana, P., Chaikuad, A., Pérez-Cuevas, E., Knapp, S., Lietha, D., Ramírez, D., Petralla, S., Monti, B., Gil, C., Martín-Requero, A., Palomo, V., De Lago, E., & Martinez, A. (2022). TDP-43 modulation by Tau-Tubulin Kinase 1 inhibitors: A new avenue for future amyotrophic lateral sclerosis therapy. *Journal of Medicinal Chemistry*, *65*, 1585–1607.
148. Taylor, L. M., Mcmillan, P. J., Liachko, N. F., Strovas, T. J., Ghetti, B., Bird, T. D., Keene, C. D., & Kraemer, B. C. (2018). Pathological phosphorylation of tau and TDP-43 by TTBK1 and TTBK2 drives neurodegeneration. *Molecular Neurodegeneration*, *13*, 7.
149. Kwon, Y. T., & Ciechanover, A. (2017). The ubiquitin code in the ubiquitin-proteasome system and autophagy. *Trends in Biochemical Sciences*, *42*, 873–886.
150. Van Eersel, J., Ke, Y. D., Gladbach, A., Bi, M., Götz, J., Kril, J. J., & Ittner, L. M. (2011). Cytoplasmic accumulation and aggregation of TDP-43 upon proteasome inhibition in cultured neurons. *PLoS ONE*, *6*, e22850.
151. Urushitani, M., Sato, T., Bamba, H., Hisa, Y., & Tooyama, I. (2010). Synergistic effect between proteasome and autophagosome in the clearance of polyubiquitinated TDP-43. *Journal of Neuroscience Research*, *88*, 784–797.
152. Scotter, E. L., Vance, C., Nishimura, A. L., Lee, Y.-B., Chen, H. J., Urwin, H., Sardone, V., Mitchell, J. C., Rogelj, B., Rubinsztein, D. C., & Shaw, C. E. (2014). Differential roles of the ubiquitin proteasome system and autophagy in the clearance of soluble and aggregated TDP-43 species. *Journal of Cell Science*, *127*, 1263–1278.
153. Dammer, E. B., Fallini, C., Gozal, Y. M., Duong, D. M., Rossoll, W., Xu, P., Lah, J. J., Levey, A. I., Peng, J., Bassell, G. J., & Seyfried, N. T. (2012). Coaggregation of RNA-binding proteins in a model of TDP-43 proteinopathy with selective RGG motif methylation and a role for RRM1 ubiquitination. *PLoS ONE*, *7*, e38658.
154. Kim, W., Bennett, E. J., Huttlin, E. L., Guo, A., Li, J., Possemato, A., Sowa, M. E., Rad, R., Rush, J., Comb, M. J., Harper, J. W., & Gygi, S. P. (2011). Systematic and quantitative assessment of the ubiquitin-modified proteome. *Molecular Cell*, *44*, 325–340.
155. Wagner, S. A., Beli, P., Weinert, B. T., Nielsen, M. L., Cox, J., Mann, M., & Choudhary, C. (2011). A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Molecular & Cellular Proteomics*, *10*, M111013284.
156. Khosravi, B., Laclair, K. D., Riemenschneider, H., Zhou, Q., Frottin, F., Mareljic, N., Czuppa, M., Farny, D., Hartmann, H., Michaelsen, M., Arzberger, T., Hartl, F. U., Hipp, M. S., & Edbauer, D. (2020). Cell-to-cell transmission of C9orf72 poly-(Gly-Ala) triggers key features of ALS/FTD. *Embo Journal*, *39*, e102811.
157. Hebron, M. L., Lonskaya, I., Sharpe, K., Weerasinghe, P. P. K., Algarzae, N. K., Shekoyan, A. R., & Moussa, C. E.-H. (2013). Parkin ubiquitinates Tar-DNA binding protein-43 (TDP-43) and promotes its cytosolic accumulation via interaction with histone deacetylase 6 (HDAC6). *Journal of Biological Chemistry*, *288*, 4103–4115.
158. Uchida, T., Tamaki, Y., Ayaki, T., Shodai, A., Kaji, S., Morimura, T., Banno, Y., Nishitsuji, K., Sakashita, N., Maki, T., Yamashita, H., Ito, H., Takahashi, R., & Urushitani, M. (2016). CUL2-mediated clearance of misfolded TDP-43 is paradoxically affected by VHL in oligodendrocytes in ALS. *Scientific Reports*, *6*, 19118.
159. Rayner, S. L., Yang, S., Farrawell, N. E., Jagaraj, C. J., Cheng, F., Davidson, J. M., Luu, L., Redondo, A. G., Rábano, A., Borrego-Hernández, D., Atkin, J. D., Morsch, M., Blair, I. P., Yerbury, J. J., Chung, R., & Lee, A. (2022). TDP-43 is a ubiquitylation substrate of the SCFcyclin F complex. *Neurobiology of Disease*, *167*, 105673.
160. Lee, Y.-C., Huang, W.-C., Lin, J.-H., Kao, T.-J., Lin, H.-C., Lee, K.-H., Lin, H.-C., Shen, C.-K. J., Chang, W.-C., & Huang, C.-C. (2018). Znf179 E3 ligase-mediated TDP-43 polyubiquitination is involved in TDP-43- ubiquitinated inclusions (UBI) (+)-related neurodegenerative pathology. *Journal of Biomedical Science*, *25*, 76.
161. Watabe, K., Kato, Y., Sakuma, M., Murata, M., Niida-Kawaguchi, M., Takemura, T., Hanagata, N., Tada, M., Kakita, A., & Shibata, N. (2020). Praja1 RING -finger E3 ubiquitin ligase suppresses neuronal cytoplasmic TDP -43 aggregate formation. *Neuropathology*, *40*, 570–586.
162. Maurel, C., Chami, A. A., Thépault, R.-A., Marouillat, S., Blasco, H., Corcia, P., Andres, C. R., & Vourc'h, P. (2020). A role for SUMOylation in the formation and cellular localization of TDP-43 aggregates in amyotrophic lateral sclerosis. *Molecular Neurobiology*, *57*, 1361–1373.
163. Maraschi, A., Gumina, V., Dragotto, J., Colombrita, C., Mompeán, M., Buratti, E., Silani, V., Feligioni, M., & Ratti, A. (2021). SUMOylation Regulates TDP-43 splicing activity and nucleocytoplasmic distribution. *Molecular Neurobiology*, *58*, 5682–5702.
164. Cohen, T. J., Hwang, A. W., Restrepo, C. R., Yuan, C.-X., Trojanowski, J. Q., & Lee, V. M. Y. (2015). An acetylation switch controls TDP-43 function and aggregation propensity. *Nature Communications*, *6*, 5845.

165. Wang, P., Wander, C. M., Yuan, C.-X., Bereman, M. S., & Cohen, T. J. (2017). Acetylation-induced TDP-43 pathology is suppressed by an HSF1-dependent chaperone program. *Nature Communications*, *8*, 82.
166. Yu, H., Lu, S., Gasior, K., Singh, D., Vazquez-Sanchez, S., Tapia, O., Toprani, D., Beccari, M. S., Yates, J. R., Da Cruz, S., Newby, J. M., Lafarga, M., Gladfelter, A. S., Villa, E., & Cleveland, D. W. (2021). HSP70 chaperones RNA-free TDP-43 into anisotropic intranuclear liquid spherical shells. *Science*, *371*, eabb4309.
167. Lu, S., Hu, J., Arogundade, O. A., Goginashvili, A., Vazquez-Sanchez, S., Diedrich, J. K., Gu, J., Blum, J., Oung, S., Ye, Q., Yu, H., Ravits, J., Liu, C., Yates, J. R., & Cleveland, D. W. (2022). Heat-shock chaperone HSPB1 regulates cytoplasmic TDP-43 phase separation and liquid-to-gel transition. *Nature Cell Biology*, *24*, 1378–1393.
168. Keating, S. S., Bademosi, A. T., San Gil, R., & Walker, A. K. (2023). Aggregation-prone TDP-43 sequesters and drives pathological transitions of free nuclear TDP-43. *Cellular and Molecular Life Sciences*, *80*, 95.
169. Igaz, L. M., Kwong, L. K., Xu, Y., Truax, A. C., Uryu, K., Neumann, M., Clark, C. M., Elman, L. B., Miller, B. L., Grossman, M., Mccluskey, L. F., Trojanowski, J. Q., & Lee, V. M.-Y. (2008). Enrichment of C-terminal fragments in TAR DNA-binding protein-43 cytoplasmic inclusions in brain but not in spinal cord of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *American Journal of Pathology*, *173*, 182–194.
170. Tsuji, H., Arai, T., Kametani, F., Nonaka, T., Yamashita, M., Suzukake, M., Hosokawa, M., Yoshida, M., Hatsuta, H., Takao, M., Saito, Y., Murayama, S., Akiyama, H., Hasegawa, M., Mann, D. M. A., & Tamaoka, A. (2012). Molecular analysis and biochemical classification of TDP-43 proteinopathy. *Brain*, *135*, 3380–3391.
171. Tsuji, H., Nonaka, T., Yamashita, M., Masuda-Suzukake, M., Kametani, F., Akiyama, H., Mann, D. M. A., Tamaoka, A., & Hasegawa, M. (2012). Epitope mapping of antibodies against TDP-43 and detection of protease-resistant fragments of pathological TDP-43 in amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *Biochemical and Biophysical Research Communications*, *417*, 116–121.
172. Nonaka, T., Kametani, F., Arai, T., Akiyama, H., & Hasegawa, M. (2009). Truncation and pathogenic mutations facilitate the formation of intracellular aggregates of TDP-43. *Human Molecular Genetics*, *18*, 3353–3364.
173. Zhang, Y.-J., Xu, Y.-F., Dickey, C. A., Buratti, E., Baralle, F., Bailey, R., Pickering-Brown, S., Dickson, D., & Petrucelli, L. (2007). Progranulin mediates caspase-dependent cleavage of TAR DNA binding protein-43. *Journal of Neuroscience*, *27*, 10530–10534.
174. Cassel, J. A., Mcdonnell, M. E., Velvadapu, V., Andrianov, V., & Reitz, A. B. (2012). Characterization of a series of 4-aminoquinolines that stimulate caspase-7 mediated cleavage of TDP-43 and inhibit its function. *Biochimie*, *94*, 1974–1981.
175. De Marco, G., Lomartire, A., Mandili, G., Lupino, E., Buccinnà, B., Ramondetti, C., Moglia, C., Novelli, F., Piccinini, M., Mostert, M., Rinaudo, M. T., Chiò, A., & Calvo, A. (2014). Reduced cellular Ca2+ availability enhances TDP-43 cleavage by apoptotic caspases. *Biochimica et Biophysica Acta*, *1843*, 725–734.
176. Li, Q., Yokoshi, M., Okada, H., & Kawahara, Y. (2015). The cleavage pattern of TDP-43 determines its rate of clearance and cytotoxicity. *Nature Communications*, *6*, 6183.
177. Dormann, D., Capell, A., Carlson, A. M., Shankaran, S. S., Rodde, R., Neumann, M., Kremmer, E., Matsuwaki, T., Yamanouchi, K., Nishihara, M., & Haass, C. (2009). Proteolytic processing of TAR DNA binding protein-43 by caspases produces C-terminal fragments with disease defining properties independent of progranulin. *Journal of Neurochemistry*, *110*, 1082–1094.
178. Suzuki, H., Lee, K., & Matsuoka, M. (2011). TDP-43-induced death is associated with altered regulation of BIM and Bcl-xL and attenuated by caspase-mediated TDP-43 cleavage. *Journal of Biological Chemistry*, *286*, 13171–13183.
179. Nishimoto, Y., Ito, D., Yagi, T., Nihei, Y., Tsunoda, Y., & Suzuki, N. (2010). Characterization of alternative isoforms and inclusion body of the TAR DNA-binding protein-43. *Journal of Biological Chemistry*, *285*, 608–619.
180. Xiao, S., Sanelli, T., Chiang, H., Sun, Y., Chakrabartty, A., Keith, J., Rogaeva, E., Zinman, L., & Robertson, J. (2015). Low molecular weight species of TDP-43 generated by abnormal splicing form inclusions in amyotrophic lateral sclerosis and result in motor neuron death. *Acta Neuropathologica*, *130*, 49–61.
181. Pesiridis, G. S., Tripathy, K., Tanik, S., Trojanowski, J. Q., & Lee, V. M.-Y. (2011). A "two-hit" hypothesis for inclusion formation by carboxyl-terminal fragments of TDP-43 protein linked to RNA depletion and impaired microtubule-dependent transport. *Journal of Biological Chemistry*, *286*, 18845–18855.
182. Sasaguri, H., Chew, J., Xu, Y.-F., Gendron, T. F., Garrett, A., Lee, C. W., Jansen-West, K., Bauer, P. O., Perkerson, E. A., Tong, J., Stetler, C., & Zhang, Y.-J. (2016). The extreme N-terminus of TDP-43 mediates the cytoplasmic aggregation of TDP-43 and associated toxicity in vivo. *Brain Research*, *1647*, 57–64.
183. Zhang, Y.-J., Caulfield, T., Xu, Y.-F., Gendron, T. F., Hubbard, J., Stetler, C., Sasaguri, H., Whitelaw, E. C., Cai, S., Lee, W. C., & Petrucelli, L. (2013). The dual functions of the extreme N-terminus of TDP-43 in regulating its biological activity and inclusion formation. *Human Molecular Genetics*, *22*, 3112–3122.
184. Berning, B. A., & Walker, A. K. (2019). The Pathobiology of TDP-43 C-Terminal Fragments in ALS and FTLD. *Frontiers in Neuroscience*, *13*, 335.
185. Chhangani, D., Martín-Peña, A., & Rincon-Limas, D. E. (2021). Molecular, functional, and pathological aspects of TDP-43 fragmentation. *Iscience*, *24*, 102459.
186. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., … Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, *596*, 583–589.
187. Takahashi, M., Kitaura, H., Kakita, A., Kakihana, T., Katsuragi, Y., Onodera, O., Iwakura, Y., Nawa, H., Komatsu, M., & Fujii, M. (2022). USP10 Inhibits Aberrant Cytoplasmic Aggregation of TDP-43 by Promoting Stress Granule Clearance. *Molecular and Cellular Biology*, *42*, e0039321.
188. Johnson, J. O., Pioro, E. P., Boehringer, A., Chia, R., Feit, H., Renton, A. E., Pliner, H. A., Abramzon, Y., Marangi, G., Winborn, B. J., Gibbs, J. R., Nalls, M. A., Morgan, S., Shoai, M., Hardy, J., Pittman, A., Orrell, R. W., Malaspina, A., … Traynor, B. J. (2014). Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. *Nature Neuroscience*, *17*, 664–666.
189. Volkening, K., Leystra-Lantz, C., Yang, W., Jaffee, H., & Strong, M. J. (2009). Tar DNA binding protein of 43 kDa (TDP-43), 14-3-3 proteins and copper/zinc superoxide dismutase (SOD1) interact to modulate NFL mRNA stability. Implications for altered RNA processing in amyotrophic lateral sclerosis (ALS). *Brain Research*, *1305*, 168–182.
190. Majumder, P., Chu, J.-F., Chatterjee, B., Swamy, K. B. S., & Shen, C.-K. J. (2016). Co-regulation of mRNA translation by TDP-43 and Fragile X Syndrome protein FMRP. *Acta Neuropathologica*, *132*, 721–738.
191. Gregory, R. I., Yan, K., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., & Shiekhattar, R. (2004). The Microprocessor complex mediates the genesis of microRNAs. *Nature*, *432*, 235–240.

192. Romano, G., Klima, R., & Feiguin, F. (2020). TDP-43 prevents retrotransposon activation in the Drosophila motor system through regulation of Dicer-2 activity. *BMC Biology*, *18*, 82.
193. Gong, D., Wang, L., Zhou, H., Gao, J., Zhang, W., & Zheng, P. (2023). Long noncoding RNA *Lnc530* localizes on R-loops and regulates R-loop formation and genomic stability in mouse embryonic stem cells. *Stem Cell Reports*, *18*, 952–968.
194. Nishimura, A. L., Župunski, V., Troakes, C., Kathe, C., Fratta, P., Howell, M., Gallo, J., Hortobágyi, T., Shaw, C. E., & Rogelj, B. (2010). Nuclear import impairment causes cytoplasmic trans-activation response DNA-binding protein accumulation and is associated with frontotemporal lobar degeneration. *Brain*, *133*, 1763–1771.
195. Gleixner, A. M., Morris Verdone, B., Otte, C. G., Anderson, E. N., Ramesh, N., Shapiro, O. R., Gale, J. R., Mauna, J. C., Mann, J. R., Copley, K. E., Daley, E. L., Ortega, J. A., Cicardi, M. E., Kiskinis, E., Kofler, J., Pandey, U. B., Trotti, D., & Donnelly, C. J. (2022). NUP62 localizes to ALS/FTLD pathological assemblies and contributes to TDP-43 insolubility. *Nature Communications*, *13*, 3380.

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