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REVIEW



# The roles of intrinsic disorder-based liquid-liquid phase transitions in the "Dr. Jekyll–Mr. Hyde" behavior of proteins involved in amyotrophic lateral sclerosis and frontotemporal lobar degeneration

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**ABSTRACT**

Pathological developments leading to amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are associated with misbehavior of several key proteins, such as SOD1 (superoxide dismutase 1), TARDBP/TDP-43, FUS, C9orf72, and dipeptide repeat proteins generated as a result of the translation of the intronic hexanucleotide expansions in the *C9orf72* gene, PFN1 (profilin 1), GLE1 (GLE1, RNA export mediator), PURA (purine rich element binding protein A), FLCN (folliculin), RBM45 (RNA binding motif protein 45), SS18L1/CREST, HNRNPA1 (heterogeneous nuclear ribonucleoprotein A1), HNRNPA2B1 (heterogeneous nuclear ribonucleoprotein A2/B1), ATXN2 (ataxin 2), MAPT (microtubule associated protein tau), and TIA1 (TIA1 cytotoxic granule associated RNA binding protein). Although these proteins are structurally and functionally different and have rather different pathological functions, they all possess some levels of intrinsic disorder and are either directly engaged in or are at least related to the physiological liquid-liquid phase transitions (LLPTs) leading to the formation of various proteinaceous membrane-less organelles (PMLOs), both normal and pathological. This review describes the normal and pathological functions of these ALS- and FTLD-related proteins, describes their major structural properties, glances at their intrinsic disorder status, and analyzes the involvement of these proteins in the formation of normal and pathological PMLOs, with the ultimate goal of better understanding the roles of LLPTs and intrinsic disorder in the "Dr. Jekyll–Mr. Hyde" behavior of those proteins.

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## 1. Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a fatal form of the neurodegeration that represents a specific neuromuscular disease characterized by the degeneration of upper and lower motor neurons of the spinal cord leading to the progressive atrophy of abdominal, bulbar, limb, and thoracic muscles.[1,2] Respiratory failure is the most common cause of death of the afflicted patients, typically within 3–5 y after onset of the ALS symptoms.[3] Despite being much less abundant than other neurodegenerative maladies, such as Alzheimer disease (AD) or Parkinson disease (PD), ALS is considered as the most common neurodegenerative disorder that affects motor neurons in adults, and is particularly infamous for its devastating physiological effects and rapid lethality. As with many other neurodegenerative diseases, most ALS cases are sporadic, and only about 8–10% of ALS cases are inherited.[1-3] As it is a neurodegenerative disorder with the progressive degeneration of motor neurons in the motor cortex of the brain, the brainstem motor nuclei, and the anterior horns of the spinal cord, progression of ALS is characterized by the spatio-temporal spread of pathology, where early stages are manifested by local symptoms, such as subtle cramping or weakness in the bulbar or limb muscles, that rapidly culminate in the global paralysis of almost all skeletal muscles and invariably lead to death.[4] The characteristic symptom of ALS is progressive amyotrophy, or muscle wasting, caused by muscle denervation; i.e., the degeneration of spinal motor neurons results in atrophy of their target muscles.[4] Since degeneration of different sets of motor neurons would affect different regions of the body, ALS is clinically characterized by its considerable heterogeneity, and multiple subsets of the disease can be distinguished. For example, based on the clinical manifestations at symptom onset, patients with sporadic ALS can be grouped into limb- and bulbar-onset subtypes, where limb onset cases are associated with the limb motor symptoms, such as weakness or atrophy or fasciculation, and where bulbar onset cases are characterized by the presence of only bulbar signs, such as dysarthria, swallowing difficulty, and tongue fasciculation.[5,6] Importantly, patients with limb- and bulbar-onset ALS subtypes are characterized by the significant difference in brain atrophy patterns and in clinical progression, which is more rapid in bulbar-onset than in limb-onset ALS.[5,7] In addition to the classical (Charcot) ALS, this spectrum of disorders includes several other syndromes, such as ALS with multisystem involvement (e.g., ALS-dementia); flail arm or Vulpian-Bernhardt syndrome; flail leg syndrome, also known as

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pseudopolyneuritic form; progressive bulbar palsy, PBP; primary lateral sclerosis, PLS; and progressive muscular atrophy, PMA.[6] Among the pathological hallmarks of ALS are shrinking and degeneration of spinal motor neurons, and accumulation of rounded or thread-like deposits of aggregated proteins (collectively known as inclusions) in the cytoplasm of affected motor neurons.[4] These inclusions are often ubiquitinated, and, in most cases of ALS, contain ubiquitinated TARDBP/TDP-43 (TAR DNA binding protein) as the major component.[8]

Because many ALS patients show cognitive impairment in addition to muscle atrophy, and develop frontotemporal lobar degeneration (FTLD, which is second to AD as a cause of dementia in patients under the age of 65, see ref. 9),[10] and because many FTLD patients show ALS symptoms,[11] it is thought now that ALS and FTLD constitute 2 parts of a clinical spectrum of a disease.[12] It was also pointed out that the overlapping pathogenesis of ALS and FTLD indicated that in these diseases, motor neuron degeneration and cognitive deficits might originate due to a common molecular basis.[2] In fact, both of these diseases are proteinopathies, because they are associated with the presence of deposits or inclusions containing various misfolded proteins, such as ATXN2 (ataxin 2); C9orf72; dipeptide repeat proteins, DPRs; FUS (FUS RNA binding protein); HNRNPA1 (heterogeneous nuclear ribonucleoprotein A1); HNRNPA2B1 (heterogeneous nuclear ribonucleoprotein A2/B1); NEK1 (NimA related kinase 1); OPTN (optineurin); SOD1 (superoxide dismutase 1); TARDBP (TAR DNA binding protein); UBQLN2 (ubiquilin 2); VCP (valosin containing protein); or members of the ubiquitin-proteasome system (UPS). Therefore, pathologically, ALS and FTLD can be grouped into multiple subtypes based on the proteins involved in the formation of corresponding inclusions. These corresponding subtypes are ALS–ATXN2, ALS–C9orf72 or ALS–DPR, ALS–FUS, ALS–HNRNPA1, ALS–HNRNPA2B1, ALS–NEK1, ALS–OPTN, ALS–SOD1, ALS–TARDBP, ALS–UBQLN2, and ALS–VCP, or FTLD–DPR, FTLD–FUS, FTLD–MAPT, FTLD–TARDBP, and FTLD–UPS.[2]

Although all aforementioned subtypes are sporadic forms of ALS and FTLD, proteinaceous inclusions of many familial forms of these diseases contain defective and mutated forms of proteins typically seen in sporadic diseases. The corresponding examples of familial ALS cases include pathologies related to the mutated or otherwise altered forms of ATXN2 (polyQ expansion), C9orf72 (hexanucleotide repeat expansions of the intronic region of the *C9orf72* gene between the noncoding exons 1a and 1b), FUS (mutations), OPTN (exon deletion, mutations), SOD1 (mutations), TARDBP (mutations), UBQLN2 (mutations), and VCP (mutations). In familial forms of FTLD, the genetic alterations include mutations in GRN/PGRN (granulin precursor) and MAPT, hexanucleotide repeat expansions near the *C9orf72* gene,[13] and mutations in several rare FTLD-related genes (or FTLD/ALS-related genes), such as CHMP2B (charged multivesicular body protein 2B); CHCHD10 (coiled-coil-helix-coiled-coil-helix domain containing 10); FUS; HNRNPA1; HNRNPA2B1; OPTN; SQSTM1 (sequestosome 1); TBK1 (TANK binding kinase 1); TARDBP; and VCP.[14] It is important to note that sporadic ALS and FTLD often arise from spontaneous mutations that were not inherited.[6,15] For example, mutations in the *TARDBP* gene have been linked to both familial and sporadic ALS.[16,17] Furthermore, increased susceptibility to sporadic ALS is thought to be associated with mutations in APOE (apolipoprotein E),[18] or in the KSP repeat region of the *NEFH* (neurofilament heavy) gene,[19,20] as well as with the decreased expression of the glutamate transporter SLC1A2/EAAT2 (solute carrier family 1 member 2)[21,22] or alterations in the *VEGF* (vascular endothelial growth factor) gene.[23] Therefore, there is a reason to think that all ALS subtypes may have a genetic root cause, whether or not familial inherited.

There are many mechanisms by which different proteins implicated in ALS or FTLD can be related to the pathogenesis of these diseases. These could be grouped into 2 major categories: loss of physiological function (the protein does not do what it is supposed to do), and gain of pathological function (the protein does what it should not do). Various factors, such as mutations, posttranslational modifications (PTMs), processing, changes in the environmental conditions (pH, temperature, ionic strength, exposure to membrane or metal ions, or some other small molecules, etc.), toxic insults, and failure of the protective system can lead to misrecognition, mistrafficking, mislocalization, misfolding, and aggregation, eventually resulting in the loss of normal functionality or the gain of pathological functions. In addition to all these factors, many of which were targets of countless studies whose outputs are covered in numerous dedicated reviews, a new threat is coming into the light of modern research, namely, the ability of some of the proteins to be engaged in the physiological liquid-liquid phase transitions (LLPTs) leading to the formation of various proteinaceous membrane-less organelles (PMLOs, such as different cytoplasmic RNA granules, including stress granules [SGs]). The goal of this review is to look into this mechanism using several illustrative examples of ALS- and FTLD-related proteins. To this end, we will first introduce some of the ALS- and FTLD-related proteins that were shown to be engaged in the LLPTs or related to the PMLOs and describe normal and pathological functions of those proteins. Then, we will consider the roles of these proteins, such as SOD1, TARDBP, FUS, C9orf72, and dipeptide repeat proteins generated as a result of the translation of the intronic hexanucleotide expansions in the *C9orf72* gene, PFN1, GLE1, PURA, FLCN, RBM45, SS18L1/CREST, HNRNPA1, HNRNPA2B1, ATXN2, MAPT, and TIA1 in the formation of PMLOs. We will also analyze a link between the normal and pathological LLPTs, and illuminate the role of liquid-liquid phase transitions and intrinsic disorder in the "Dr. Jekyll–Mr. Hyde" behavior of these proteins.

## 2. Intrinsic disorder in ALS- and FTLD-related proteins

### 2.1. A brief overview of the intrinsic disorder phenomenon: What are the intrinsically disordered proteins and what are they for?

The fact that numerous biologically active protein regions and even entire proteins do not possess stable tertiary and/or secondary structures is rapidly becoming the new reality in protein science. These naturally flexible proteins are known now as intrinsically disordered proteins (IDPs),[24] a term selected by the scientific community from several other names used in the

early literature dealing with this topic (e.g., natively denatured,[25] natively unfolded,[26] intrinsically unstructured,[27] and natively disordered proteins,[28] among many other terms). In addition to proteins that are intrinsically disordered as a whole, a much larger cohort of proteins is described as hybrid proteins that contain both ordered domains and IDP regions (IDPRs).[29] These proteins/regions exist as highly dynamic structural ensembles possessing multiple rapidly interconverting conformations.[30,31] The IDPRs are characterized by remarkable spatiotemporal heterogeneity, and their structures can range from expanded, random coil-like ensembles, to pre-molten globule-like ensembles, which are the highly disordered entities containing some localized regions of transient secondary structure, to molten globule-like species with well-developed secondary structure and mobile side-chains.[32–34]

There are multiple levels at which IDPs and IDPRs differ from structured globular proteins and domains. Such differences can be found in amino acid composition, charge, flexibility hydrophobicity, and sequence complexity. For example, IDPs/IDPRs are significantly depleted in so-called order-promoting residues (I, L, V, W, F, Y, C, and N), and contain more disorder-promoting residues, such as A, R, G, Q, S, P, E, and K.[24,35–37] Based on these and other remarkable differences between the amino acid sequences of ordered proteins/domains on the one side and IDPs/IDPRs on the other side, numerous disorder predictors were developed. A short list of commonly used predictors include members of the PONDR family,[35,38] CH-plot,[39] NORSp,[40] GlobPlot,[41,42] FoldIndex,[43] IUPred,[44] DisoPred,[45–47] DisCon,[48] MFDp2,[49] and many others. It was pointed out that, because different computational tools use different sequence attributes and different definitions of disorder, additional important information related to the peculiarities of predicted disorder can be retrieved via comparing and combining the results of several predictors on an individual protein of interest or on a protein dataset.[50-57] Furthermore, several methods were developed for the prediction of disordered protein binding regions including alpha-MoRF-Pred,[58] ANCHOR,[59] MoRFpred,[60] PepBindPred,[61] MFSPSSMpred,[62] DISOPRED3,[63] MoRFCHiBi,[64] fMoRFpred,[65] and DisoRDPbind,[66,67] indicating that functions of IDPRs and IDPs are predictable from protein sequences. Finally, because sites of the enzyme-catalyzed posttranslational modifications, such as phosphorylation,[68] acetylation, methylation, and ubiquitination[69] are commonly located within the IDPRs, several computational tools utilizing this information have been developed, such as DisPhos (disorder-enhanced phosphorylation predictor), which can efficiently find IDPR-located phosphorylation sites with 76% accuracy for serine, 81% for threonine, and 83% for tyrosine.[68] More recently, another tool has been developed which is a unified sequence-based predictor of 23 types of post-translational modification (PTM) sites.[69]

The high abundance of intrinsic disorder in nature was illustrated in several computational studies of different proteomes where various disorder predictors were utilized. These same studies showed that the overall level of disorder of proteins increases with organism complexity, and that over half of eukaryotic proteins are predicted to have long IDPRs.[47,50,70,71]

The conformational adaptability and structural plasticity of IDPs/IDPRs, their ability to quickly react in response to the changes in their environment and to fold under a variety of conditions,[24,27,39,72–81] combined with their unique capabilities to fold differently while interacting with different binding partners and to be able to engaged in promiscuous binding,[78,82] define a wide set of functions exerted by IDPs/IDPRs in different biological systems, and explain and determine the broad participation of IDPs/IDPRs in various biological processes,[34,83,84] where they can be involved in various signaling processes,[85,86] regulation of numerous pathways,[87–94] cell protection,[95] protein protection,[96,97] cellular homeostasis,[98,99] and controlled cell death.[100–104]

These same structural features also define the ability of IDPs to modulate and control functions of their binding partners and to promote and coordinate the assembly of various complexes.[105] IDPs can be engaged in a wide spectrum of interactions, ranging from those leading to the formation of highly stable assemblies to weak but specific signaling interactions.[32] It is also important to remember that although binding of IDPs/IDPRs to their partners is often accompanied by the disorder-to-order transitions, many IDPs and IDPRs preserve significant amounts of disorder in their bound states,[32] leading to the formation of so-called disordered, dynamic, or fuzzy complexes, being bound to ordered proteins,[106–111] other disordered proteins,[112–114] membranes,[115,116] or nucleic acids. Overall, IDPs/IDPRs can form static, semi-static, and dynamic or fuzzy complexes.[33] Static and semi-static binding modes range from the interaction-induced folding of a whole molecule to binding-triggered gaining of local structure on the surface of a binding partner, and from penetrating deep inside the binding partner to wrapping around the binding partner.[117] IDPs can participate in one-to-many and many-to-one interactions, where one IDPR binds to multiple partners potentially gaining very different structures in the bound state, or where multiple unrelated IDPs/IDPRs bind to one partner.[32]

Furthermore, there are several means by which biological activities of IDPs/IDPRs are tightly controlled and regulated in a precise and timely manner. These means include alternative splicing generating multiple protein isoforms with highly diverse regulatory elements[118–120] and various PTMs, such as acetylation, glycosylation, phosphorylation, etc.[34,69,121,122] In fact, the multifunctionality of IDPs/IDPRs relies (at least in part) on the presence of multiple relatively short functional elements that are spread within their amino acid sequences[32] and can be dramatically reshuffled by alternative splicing (AS),[119,120,123] which can generate a set of protein isoforms with a highly diverse set of regulatory elements.[118]

### 2.2. Proteoforms: Intrinsic disorder and one sequence–many functions paradigm

The functional multifariousness of IDPs/IDPRs represents an important illustration for the paramount conclusion that the proteome size, not the genome size, defines the complexity of a biological system.[124] This is because the number of functionally divergent proteins is typically higher than the number of genes. For example, in a human cell, the number of protein-coding genes approaches 21,000,[125] while the number of functionally different proteins is substantially higher (in the range of a few hundred thousand, if not millions).[126–130] There are several mechanisms that contribute to this functional diversification of

proteins and can affect a given gene product at the DNA, mRNA, or protein levels. Among such diversifiers are single or multiple point mutations, indels (i.e., insertions or deletions), and single nucleotide polymorphisms causing allelic variations contributing at the DNA level; AS, mRNA editing, the use of alternative promoters, alternative initiation of translation, and other pre-translational mechanisms affecting mRNA; and different PTMs that can change physico-chemical properties of a polypeptide chain. Therefore, instead of the "one gene–one protein–one function" view, the actual gene-protein relationship is much more complex, being better described by the "one gene–many proteins–many functions" model (see Fig. 1).

In other words, there are various means that ensure creation of a wide spectrum of rather different protein molecules from a single gene. This multilevel diversification of gene products is at the root of the proteoform concept,[131] where proteoforms "designate all of the different molecular forms in which the protein product of a single gene can be found, including changes due to genetic variations, alternatively spliced mRNA, and PTMs".[131] Subsequently, it was pointed out that, in addition to the means for increasing the chemical variability of a polypeptide chain, the protein structural and functional diversity can be further extended via protein intrinsic disorder and function itself.[132–134] Here, any given protein can be considered as a basic (or intrinsic, or conformational) proteoform because it does exist as a dynamic conformational ensemble, members of which have different structures and can potentially have different functions. There are also inducible proteoforms generated by various allelic-, mRNA-, and PTM-based diversifications of the canonical protein sequence. Finally, because structural ensembles of both basic and induced proteoforms can be affected by protein function, interaction with specific partners, or placement inside the natural cellular environment (which is extremely crowded, characterized by the presence of high concentrations of various biological macromolecules,[135-137] has limited available volume,[138] and considerably restricted amounts of free water[135,139–143]), protein functionality can produce functioning proteoforms. Obviously, any member of the inducible (or modified) proteoform (i.e., any mutated, modified, or alternatively spliced form) or functioning proteoform is a conformational proteoform itself because it also represents a structural ensemble.[132–134] Curiously, alternative splicing generating multiple protein isoforms[118–120] and various enzymatically catalyzed PTMs, such as acetylation, glycosylation, phosphorylation, etc.,[34,69,121,122] are commonly located within the regions with intrinsic disorder, providing additional link between conformational/basic and inducible/modified proteoforms.

All these considerations, taken together, define the ability of a protein to have a multitude of structurally and functionally different states, which are constituents of the "protein structure-function continuum", where a given protein exists as a dynamic conformational ensemble containing multiple proteoforms (conformational/basic, inducible/modified, and functioning) characterized by diverse structural and functional features.[132–134]

### 2.3. Protein intrinsic disorder and human diseases

Since IDPs/IDPRs are common universal regulators and controllers, they are tightly controlled and regulated[144] via multiple mechanisms, such as interaction with chaperones[145–149] or nanny proteins,[150] binding to specific partners of proteinaceous and non-proteinaceous nature,[151–157] various PTMs,[87,158–161]

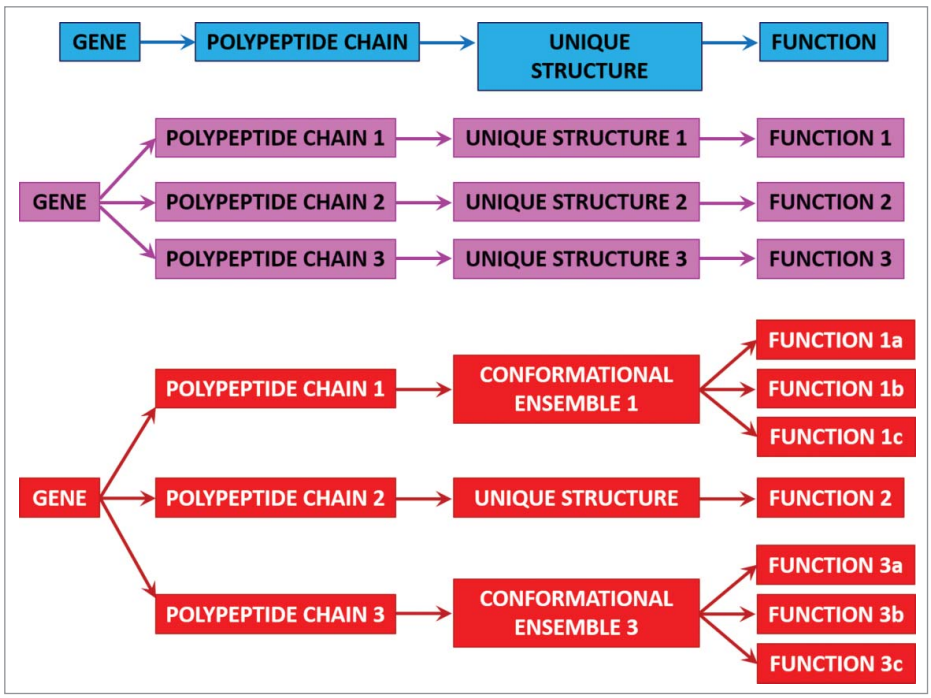


**Figure 1.** Classic and modern protein structure-function relationships. Schematic representation of the classic "one gene–one protein–one function" paradigm (top part, blue) and its modification by alternative splicing and PTMs when affected genes encode ordered proteins (middle part, pink) or intrinsically disordered and hybrid proteins containing ordered and intrinsically disordered domains (bottom part, red). Reproduced with permission from ref. 132.

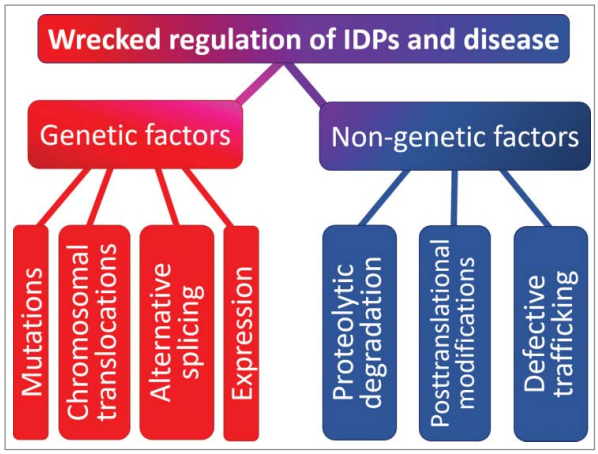


**Figure 2.** Factors causing pathogenic transformation in an IDP/IDPR. Schematic representation of different ways by which aberrant regulation at the genetic level or various posttranslational (non-genetic) mechanisms can cause pathogenic transformation in an IDP/IDPR. Reproduced with permission from ref. 171.

and regulated degradation.[162–167] As the consequences of misbehavior of an important controller (reflected in the inability of such a protein to adopt and keep a functional state) or any distortion in the tightly controlled processes could be disastrous, it is not surprising that the development of particular pathological conditions is frequently associated with the dysfunction of IDPs manifested in protein misfolding and aggregation leading to the loss of normal function and gain of toxic function.[168–170] Fig. 2 represents some of the factors related to this phenomenon and shows that the aberrant regulation at the genetic level or various posttranslational (nongenetic) mechanisms can cause pathogenic transformation in an IDP/IDPR.[171] The complexity of this picture, where numerous factors contribute to conformational diseases, is further increased by the ability of all these factors to not only work independently, but to also take action additively or synergistically.[171,172] Therefore, many IDPs and hybrid proteins contacting ordered domains and long IDPRs are related to the development of various diseases.[169,173,174] For example, in addition to the usual suspect, TP53/p53 (tumor protein p53),[175] some other well-known cancer-related proteins with experimentally confirmed IDPRs include APC (adenomatous polyposis coli),[176,177] TP53BPs/ASPPs (tumor protein p53 binding protein),[178,179] AXIN1 (axin 1),[176,180,181] BH3-only proteins,[182] BRCA1 (BRCA1, DNA repair associated),[183] CTAs/cancer-testis antigens,[184] CREBBP/CBP (CREB binding protein) and its paralog, EP300/p300 (E1A binding protein p300),[156] EWSR1 (EWS RNA binding protein 1),[185] proteins of human papillomavirus (HPV),[186] PTEN (phosphatase and tensin homolog),[187] CDKN1A/p21 (cyclin-dependent kinase inhibitor 1A) and CDKN1B/p27Kip1 (cyclin-dependent kinase inhibitor 1B),[92] SIRTs/NAD-dependent protein deacetylase sirtuins,[188,189] and many others. Many of the neurodegeneration-related proteins are also IDPs. Illustrative examples of highly disordered proteins engaged in various neurodegenerative diseases include a protein-chameleon, SNCA/α-synuclein,[190–195] associated with PD, dementia with Lewy bodies, AD, Down syndrome, and several other synucleinopathies, ataxin in spinocerebellar ataxia, prions in Creutzfeldt-Jakob disease and transmissible spongiform encephalopathies, amyloid β in AD, and MAPT in AD and many other tauopathies.[168,196] Among other human diseases for which involvement of IDPs was demonstrated are AIDS (HIV Rev protein),[197] cardiovascular disease (e.g., F2 [coagulation factor II, thrombin]),[198] cystic fibrosis (CFTR [cystic fibrosis transmembrane conductance regulator]),[199] and type II diabetes (IAPP/amylin [islet amyloid polypeptide]).[173]

### 2.4. Intrinsic disorder in proteins related to ALS and FTLD

Fig. 3 demonstrates that all ALS- and FTLD-related proteins considered in this review contain significant levels of intrinsic disorder. The analogous results for the dipeptide repeat proteins poly(GA), poly(GR), poly(GP), poly(PA), and poly(PR) synthesized as a result of the repeat-associated non-ATG (RAN) translation of the sense and antisense repeat RNAs derived from the intronic region of the *C9orf72* gene are not shown in this figure. This is because of the well-known fact that the repeat-containing proteins are often intrinsically disordered, with the more perfect repeats being less structured.[200] Therefore, it is expected that these proteins should be mostly disordered, and in agreement with this expectation, all these proteins were predicted to be highly disordered.[201]

Results shown in Fig. 3 are in agreement with the results reported in previous studies. In fact, prevalence of intrinsic disorder in proteins associated with various neurodegenerative diseases was the subject of several dedicated studies.[168,170,171,196,201–203] Also, some proteins associated with biological liquid-liquid phase transitions (LLPTs) and formation of various proteinaceous membrane-less organelles (PMLOs), including stress granules (SGs), are disordered.[204–206] As a result, in those studies, the intrinsic disorder status and biological roles of IDPRs were already systemized for several proteins considered in this review (SOD1, TARDBP, FUS, C9orf72 and DPRs, PFN1, TIA1, MAPT, HNRNPA1, HNRNPA2B1, and ATXN2).

Because interested readers can find details of those analyses in the corresponding publications,[168,196,205,206] there is no need to reproduce them here. Therefore, this section represents some data on the intrinsic disorder status of the remaining ALS-FTLD-related proteins that also play important roles in SG formation (GLE1, PURA, FLCN, RBM45, and SS18L1). It is important to keep in mind that the in-depth analysis of the intrinsic disorder predispositions of all these proteins and the detailed consideration of the potential roles of their IDPRs is outside the scope of this article.

***Nucleoporin GLE1*** (GLE1, RNA export mediator; UniProt ID: Q53GS7) is the nucleoporin responsible for the export of the poly(A)-tail containing mRNAs from the nucleus into the cytoplasm[207–210] and is also known as a multifunctional regulator of gene expression.[211] The N-terminal region (residues 1–30) of human GLE1 is required for interaction with the nuclear pore complex via the nucleoporin NUP155 (nucloporin 155),[212] whereas the coiled-coil domain containing 2 coiled-coil regions is required for GLE1 self-oligomerization and localization to the nuclear pore complex.[210] Fig. 3F shows that the N-terminal half of this protein containing these functionally important regions is predicted to be mostly disordered.

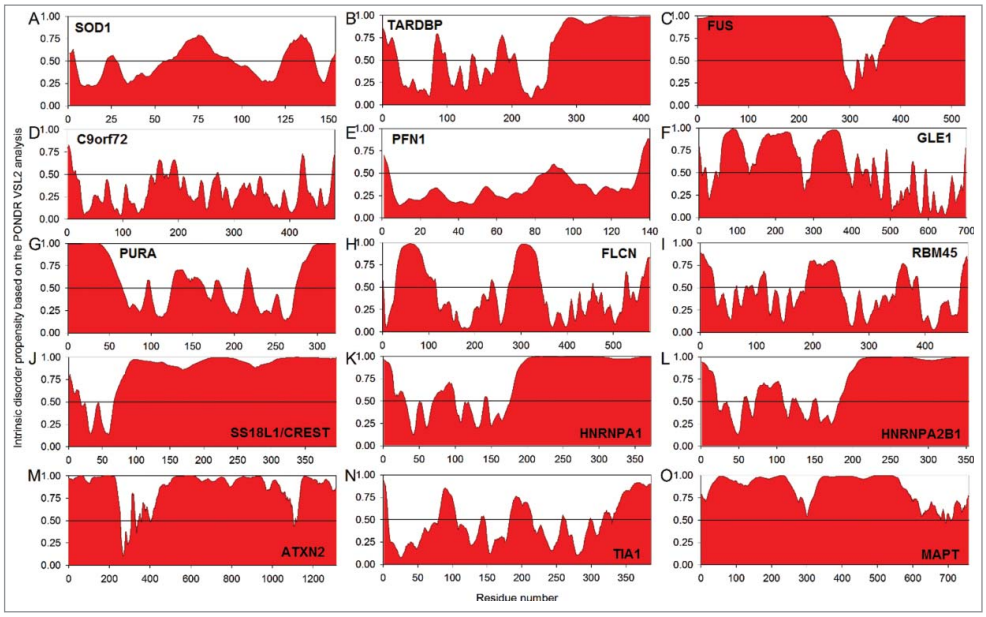


**Figure 3.** Intrinsic disorder in proteins presented in this study. The intrinsic disorder predisposition of these proteins was evaluated by PONDR® VSL2. (A) SOD1; (B) TARDBP; (C) FUS; (D) C9orf72; (E) PFN1; (F) GLE1; (G) PURA; (H) FLCN; (I) RBM45; (J) SS18L1/CREST; (K) HNRNPA1; (L) HNRNPA2B1; (M) ATXN2; (N) TIA1; and (O) MAPT. In the corresponding plots, regions with disorder scores above the 0.5 threshold (shown as a thin black line) are predicted to be intrinsically disordered.

A high level of disorder in this protein is also confirmed by the MobiDB database (http://mobidb.bio.unipd.it/)[213,214] which generates consensus disorder scores for a query protein by aggregating the output from 10 predictors. Such consensus MobiDB disorder content of human GLE1 is 25.50%. The conclusion on the presence of high levels of disorder in human GLE1 protein is further supported by Fig. 4A, which shows the results of the analysis of this protein using the $D^2P^2$ database (http://d2p2.pro/)[215] that provides disorder evaluations by several computational tools and also shows some important disorder-related functional information, such as location of various curated PTMs and ANCHOR-predicted disorder-based

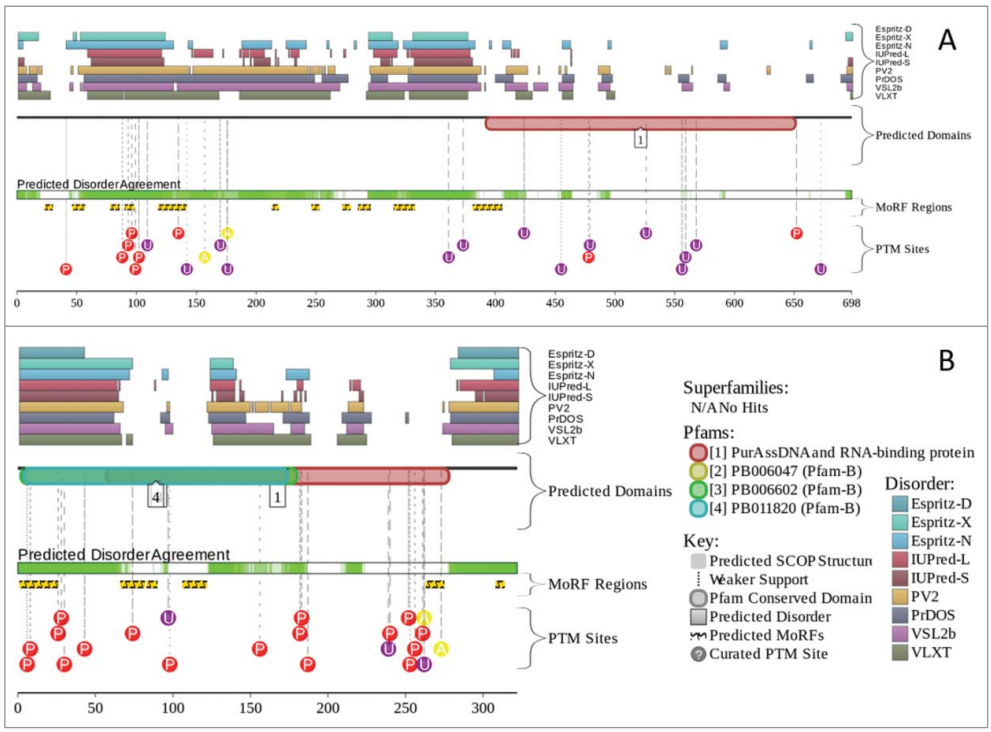


**Figure 4.** Multifactorial analysis of intrinsic disorder in GLE1 (UniProt ID: Q53GS7) (A) and PURA (UniProt ID: Q00577) (B). Intrinsic disorder propensity and some important disorder-related functional information generated for the corresponding proteins by the $D^2P^2$ database are shown. Here, the green-and-white bar in the middle of the plot shows the predicted disorder agreement among 9 predictors, with green parts corresponding to disordered regions by consensus. The yellow bar shows the location of the predicted disorder-based binding sites (molecular recognition features, MoRFs), whereas colored circles at the bottom of the plot show the location of various PTMs.

protein-protein interaction sites,[59,216] known as molecular recognition features, MoRFs (see 58, 217–219). Fig. 4A shows that human GLE1 is predicted to have 11 MoRFs, all located within the N-terminal half of the protein, and a multitude of PTMs, such as phosphorylation, ubiquitination, and acetylation. All this clearly indicates that abundant disorder serves several important functions for this protein.

***Transcriptional Activator PURA/Pur-α*** (UniProt ID: Q00577; MobiDB consensus disorder content is 41.93%) is a highly conserved multifunctional single-stranded DNA- and RNA-binding protein[220] that serves as a transcriptional activator. Fig. 3G and 4B show that PURA is predicted to have intrinsically disordered N- and C-terminal tails and also has a disordered region in the middle of the sequence. It is predicted to have 6 MoRFs and several phosphorylation, ubiquitination, and acetylation sites spread throughout the sequence of this protein (Fig. 4B).

***FLCN (folliculin*;** UniProt ID: Q8NFG4; MobiDB consensus disorder content is 23.32%) is a tumor suppressor, that is predicted to contain 2 long IDPs and a disordered C-tail (Fig. 3H and 5A). The centrally located disordered region contains the coiled-coil domain (residues 287–310). FLCN is predicted to have 2 MoRFs located within the N-terminal IDPR, as well as several sites of phosphorylation and ubiquitination (Fig. 5A).

***SS18L1/CREST*** (UniProt ID: O75177; MobiDB consensus disorder content is 86.16%) is a 396-residue-long, highly disordered transcription activator that has a disordered N-tail, 1 ordered domain that contains 2 MoRFs, and a long, highly disordered region covering the central and C-terminal parts of this protein (Fig. 3J and 5B). This disordered region serves as an excessive binding platform, because 11 of 13 MoRFs predicted in SS18L1 are located there. Overall, Fig. 5B shows that 55.6% of human SS18L1 residues are expected to be engaged in disorder-based protein-protein interactions.

***RBM45*** (***RNA binding motif protein 45***; UniProt ID: Q8IUH3; MobiDB consensus disorder content is 18.07%) is an RNA-binding protein with binding specificity for poly(C). Although there is no information about this protein in $D^2P^2$, a multiparametric analysis of this protein by PONDR® VLXT, PONDR® FIT, PONDR® VL3, PONDR® VSL2, IUPred_short,

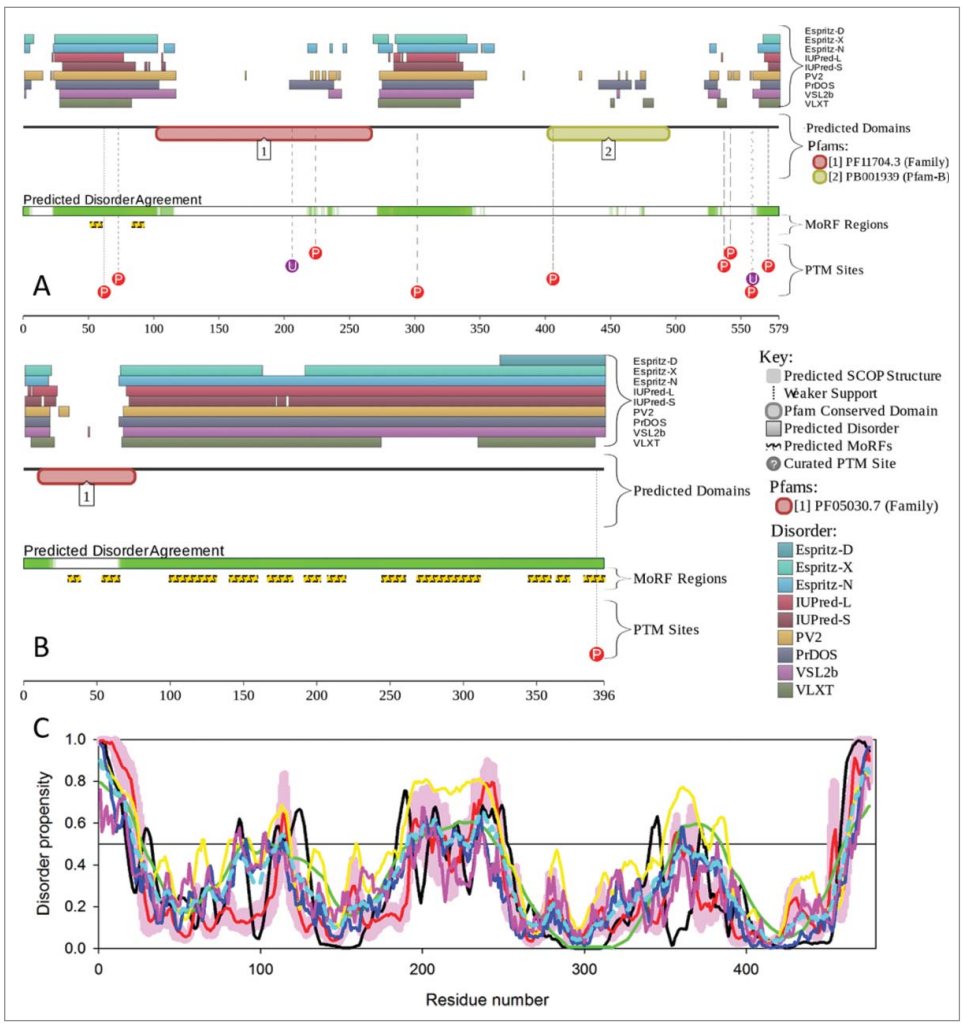


**Figure 5.** Multifactorial analysis of intrinsic disorder in FLCN (UniProt ID: Q8NFG4) (A), SS18L1/CREST (UniProt ID: O75177) (B), and RBM45 (UniProt ID: Q8IUH3) (C). Plots (A) and (B) were generated using the $D^2P^2$ database. Here, keys are similar to those described in legends to Fig. 4. Because the $D^2P^2$ database does not have related information for human RBM45, plot (C) represents disorder profiles generated by PONDR® VLXT (black line), PONDR® FIT (red line), PONDR® VL3 (green line), PONDR® VSL2 (yellow line), IUPred_short (blue line) and IUPred_long (pink line). The cyan dashed line shows the mean disorder propensity calculated by averaging the disorder profiles of the individual predictors. The light pink shadow around the PONDR® FIT shows the error distribution. In these analyses, the predicted intrinsic disorder scores above 0.5 are considered to correspond to the disordered residues/regions, whereas regions with disorder scores between 0.2 and 0.5 are considered flexible.

and IUPred_long shown in Fig. 5C suggests that this protein contains several disordered and flexible regions; that is, regions with disorder scores exceeding 0.5 and ranging between 0.2 and 0.5, respectively. Also, there are 3 MoRFs in human RBM45.

## 3. Stress granules, other proteinaceous membrane-less organelles, and biological liquid-liquid phase transitions

Colocalization of biological macromolecules (proteins and nucleic acids) at high concentrations within a small cellular micro-domain leads to the formation of various proteinaceous membrane-less organelles (PMLOs), including stress granules (SGs). These PMLOs are highly dynamic assemblages, which, due to the presence of various RNAs and proteins, are generally known as ribonucleoprotein (RNP) granules, RNP bodies, or RNP droplets.[221] Although such PMLOs can typically be found in the cytoplasm and the nucleoplasm of eukaryotic cells, they are also present in mitochondria and, in plants, in chloroplasts (Fig. 6). As a matter of fact, the known set of PMLOs exceeds 25 species. The incomplete list of cytoplasmic PMLOs includes centrosomes,[222] germline P-granules (germ cell granules or nuage),[223,224] neuronal RNA granules,[225] processing bodies or P-bodies,[226] and stress granules.[227] Most commonly known nuclear PMLOs are Cajal bodies,[228] chromatin,[229] cleavage bodies,[230] histone locus bodies,[231] nuclear gems (Gemini of Cajal bodies),[232,233] nuclear pores,[234] nuclear speckles or interchromatin granule clusters,[235] nuclear stress bodies,[236,237] nucleoli,[238] Oct1/PTF/ transcription (OPT) domains,[239] paraspeckles,[240] perinucleolar compartment,[241] PcG bodies (polycomb bodies, subnuclear organelles containing polycomb group proteins),[242] promyelocytic leukemia nuclear bodies or nuclear dots,[243] and the Sam68 nuclear body.[241] Mitochondria and chloroplasts have RNA granules[244] and chloroplast SGs, respectively.[245] This realm of cytoplasmic, nuclear, mitochondrial and chloroplast PMLOs is depicted in Fig. 6. One should keep in mind that although in this review SGs are the most frequently discussed PMLOs, pathological cellular transformations may also engage other organelles (e.g., P-bodies, neuronal RNA granules, CREBBP-containing nuclear bodies, and paraspeckles among others, see below).

The important feature of PMLOs that clearly differentiate them from other cellular organelles is the fact that, contrary to the "traditional" membrane-encapsulated organelles, the structural integrity of PMLOs is not supported by encapsulation in the membrane. Instead, their biogenesis is entirely controlled and mediated via a multitude of protein-protein, protein-RNA, and/or protein-DNA interactions.[246] Furthermore, this lack of membrane encapsulation defines the liquid-like behavior, such as dripping, relaxation to spherical structures upon fusion, and wetting, commonly described for various PMLOs.[223,227,247,248] Also, the lack of encapsulation defines the highly dynamic nature of PMLOs and ensures the direct contact of their components with the surrounding cytoplasm or nucleoplasm.[249,250] Based on all these considerations, as well as on the fact that PMLOs are just slightly denser than the rest of the cytoplasm or nucleoplasm[251,252] and possess high level of internal dynamics, these organelles are defined as liquid-droplet phases of the nucleoplasm/cytoplasm.[223,227,247,248,253,254] Curiously, although PMLOs are considered as a different liquid state of the cytoplasm or nucleoplasm, their major biophysical properties are rather similar to those of the rest of the intracellular fluid.[221] PMLOs have unique morphologies, specific distribution patterns, and, at the molecular level, their structures are determined by a specific set of resident proteins.

It is assumed that the formation of SGs and other PMLOs represents a result of the liquid-liquid phase transition (or fluid-fluid demixing).[221,223,247,255–258] Biophysically, besides the obvious requirement of the presence of high enough concentrations of the related constituents compartmentalized within a limited enough volume, the efficiency of such LLPTs depends on the flexibility and multivalency of the participating components and on the ability of these constituents to be engaged in multiple highly controllable low-affinity interactions.[253,259–270] It was emphasized that highly dynamic structures, a low

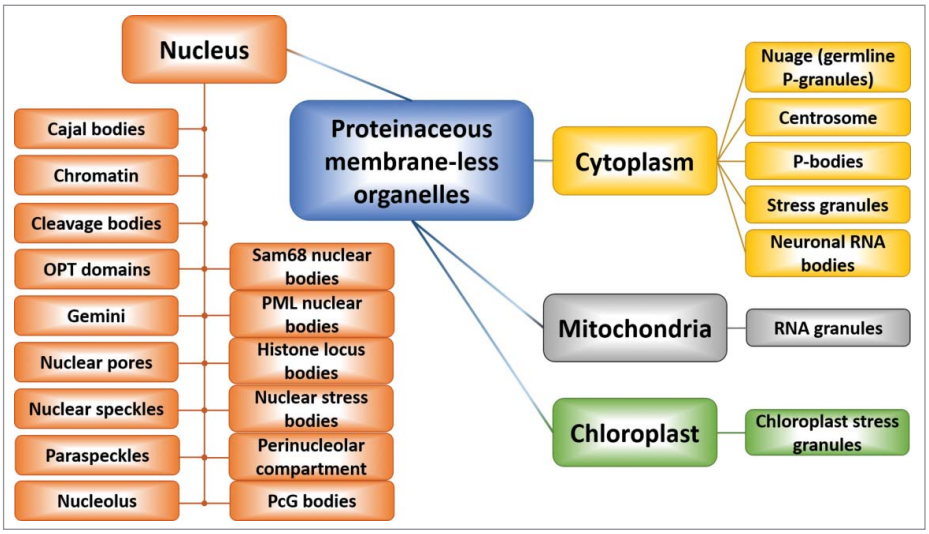


**Figure 6.** Diversity of PMLOs found in eukaryotic cells. Schematic representation of the multitude of cytoplasmic, nuclear, mitochondrial and chloroplast PMLOs.

complexity of amino acid sequences that can be considered as a string of repetitive amino acids or groups of amino acids, and the ability to be engaged in multiple low-affinity interactions with other proteins and nucleic acids of IDPs and IDPRs, make them ideal drivers of such LLPTs, and, in agreement with this hypothesis, many PMLOs abundantly contain IDPs/IDPRs.[205,206,271–273]

Since SGs are the PMLOs which are most frequently considered in association with ALS, these RNP droplets are discussed below in greater detail. Readers interested in other PMLOs are can peruse recent reviews.[206,221,273,274] As with all other PMLOs, SGs are complex assemblages that include proteinaceous and RNA constituents and are typically formed as a specific cellular response to stressful conditions or to a variety of environmental and cellular cues. SGs include components of the translation-competent 48S complex (eukaryotic initiation factors EIF3 complex, EIF4G1/EIF4F, EIF4B, the small ribosomal subunits, PABPC1/PABP1 (poly[A] binding protein cytoplasmic 1), and stalled mRNA transcripts),[275] many other RNA binding proteins (RBPs; primary SG formers) that are involved and regulate mRNA stabilization, processing, translation, and transport,[276] and, being overproduced, are able to induce SG formation in the absence of stress (such as PABPC1, TIA1, TIA1L/TIAR [TIA1 cytotoxic granule associated RNA binding protein like 1], G3BP1 [G3BP stress granule assembly factor 1], CPEB [cytoplasmic polyadenylation element binding protein], FXR1 FMR1 autosomal homolog 1], FMR1/FMRP [fragile X mental retardation 1], and CASC3/MLN51 [cancer susceptibility 3]),[276–283] as well as a variety of other proteins that are recruited to the SGs via protein-protein interactions with primary SG formers.[275] Furthermore, these cytoplasmic foci contain various mRNA stalled at the pre-initiation stage and can therefore be considered as cytoplasmic messenger ribonucleoprotein particles that possess defined cytoprotective functions.[283] Among the biological functions ascribed to SGs are modification of the local patterns of translation within the cell, sequestration of various signaling molecules that regulate cell viability, and sequestration and silencing of mRNAs encoding house-keeping proteins.[275,284] Furthermore, under stress conditions SGs participate in shifting RNA translation towards cytoprotective proteins, such as chaperones and heat shock proteins.[285] The list of regulatory proteins sequestered by SGs includes several regulators of apoptosis, such as RACK1 (receptor for activated protein C kinase 1), TRAF2 (TNF receptor-associated factor 2),[281,286,287] and MTORC1 (mechanistic target of rapamycin complex 1) that serves as a regulator of cell growth and metabolism.[288,289]

Of special importance is the fact that, unlike amyloid or amyloid-like fibrillation, the process of SG formation is completely reversible and is tightly controlled.[290] The formation of SGs, being an illustrative example of biological LLPT, is a very complex process. In brief, SG nucleation is initiated by several RNA-binding proteins, with the most commonly examined being TIA1, TIAL1, ZFP36/TTP (ZFP36 ring finger protein), and G3BP,[275] whereas SG maturation involves incorporation of many pro-apoptotic proteins (e.g., TRAF2, ROCK1 (Rho associated coiled-coil containing protein kinase 1), and RACK1 and other regulators of apoptosis (such as RPS6KA3/RSK2 [ribosomal protein S6 kinase A3] and FASTK [Fas activated serine/threonine kinase]), leading to the inhibition of the apoptotic response.[290] Among apoptosis-unrelated signaling and regulating proteins incorporated into the SGs are MAPK8/JNK1 (mitogen-activated protein kinase 8), MAP2K7/MKK7 (mitogen-activated protein kinase kinase 7), RHOA (ras homolog family member A), AKAP9/AKAP350 (A-kinase anchor protein 9), WDR62 (WD repeat domain 62), and HDAC6 (histone deacetylase 6).[290]

An interesting recent development is the recognition of the importance of SGs for the pathology of several neurodegenerative diseases,[283,290–292] including ALS and FTLD.[283,293-299] Relation of SGs to neurodegeneration comes from the following observations:

(i) SG marker proteins, such as PABPC1 and G3BP, are found in cytosolic inclusion bodies of patients with AD, ALS, and FTLD, indicating that distorted SG dynamics might contribute to pathogenesis of these and other neurodegenerative diseases[300];
(ii) in many neurodegenerative diseases, there is an obvious colocalization of SGs with insoluble protein aggregates[290];
(iii) SGs frequently contain RNA-binding proteins related to the pathogenesis of various neurodegenerative diseases, such as TARDBP and FUS, related to the pathology of ALS and FTLD, SMN (survival motor neuron protein) related to the spinal muscular atrophy pathology, spinocerebellar ataxia 2-related ATXN2, OPTN related to primary open angle glaucoma and amyotrophic lateral sclerosis 12, and ANG (angiogenin) that is involved in amyotrophic lateral sclerosis 9 pathology;
(iv) Mutations in the synaptic functional regulator FMR1, which is a protein that plays a central role in neuronal development and synaptic plasticity through the regulation of alternative mRNA splicing, mRNA stability, mRNA dendritic transport, and postsynaptic local protein synthesis of a subset of mRNAs[301–305] and is associated with fragile X syndrome) or HNRNPA1 (an RNA-binding protein related to ALS-FTLD pathogenesis) cause severe impairment of SG dynamics[300];
(v) also, MAPT,[290] PRNP/ PrP (prion protein),[306] HTT (huntingtin),[307] and some other Q/N-rich proteins[308] are associated with SGs and modulate SG formation.

The roles of various proteins related to ALS and/or FTLD and involved in SG formation are considered below.

## 4. Normal and pathological functions of the ALS- and FTLD-related proteins and their roles in biogenesis of stress granules and other PMLOs

### 4.1. SOD1/Cu-Zn superoxide dismutase 1

#### 4.1.1. Structural properties of SOD1

The *SOD1* gene is located at position 22.11 within the long (q) arm of chromosome 21 (genetic location 21q22.11), and its coding region consists of 5 exons interrupted by 4 introns.[309] Struturally, human SOD1 (UniProt ID: P00441) is a homodimer whose protomers are held together by hydrophobic

interactions.[310,311] The structure of this protein is characterized by the highly conserved topology found in all the intracellular eukaryotic SODs, where each protomer has 153 amino acids arranged in a Greek key $\beta$-barrel structure composed of 8 antiparallel $\beta$-strands.[312] Homodimeric SOD1 is characterized by a very high conformational stability, with the melting temperature ($T_m$) exceeding 90°C.[313] This high stability is determined by 3 factors associated with the posttranslational maturation of this protein, such as the coordination of 1 $Zn^{2+}$ and 1 $Cu^{2+}$ ion by each protomer of the homodimer, the formation of the intrachain disulfide bond Cys58-Cys147, and dimerization. In fact, disulfide-reduced apo-SOD1 (i.e, a protein with metal ions removed) has a $T_m$ of ~42°C,[314,315] which makes it highly susceptible for spontaneous partial unfolding followed by misfolding and aggregation under physiological conditions. Amyloid-like fibrils, which are a pathological hallmark in SOD1-related patients with familial ALS,[316] are formed only by the disulfide-reduced apo-SOD1.[317] This clearly indicates that intracellular deregulation of metal binding and/or disulfide formation might be among the key factors triggering the pathological misfolding of SOD1.[318] In agreement with this hypothesis, many ALS-related mutations of SOD1 affect the post-translational control of SOD1 maturation,[319,320] leading to an increase in the intracellular populations of the apo-,[321] and/or disulfide-reduced forms of this protein.[322]

Reduction of the disulfide bond causes increased disorder in the apo-SOD1 generating a conformation significantly different from the structure of the wild-type SOD1 (WT-SOD1) protomer.[318] These observations are in strong agreement with a systematic computational analysis of the folding and structural dynamics of monomeric and dimeric forms of SOD1 in their holo- and apo-forms, both in the disulfide-intact and disulfide-reduced states by the all-atom discrete molecular dynamics (DMD) simulations, which revealed that although the disulfide bond formation has less pronounced stabilizing effects than the metal binding, the disulfide bond nevertheless has a significant contribution to protein dynamics.[323] It was also shown that the reduction of this disulfide bond is necessary for initiation of fibril formation.[324]

#### 4.1.2. Normal and pathological functions of SOD1

SOD1 is an antioxidant metalloenzyme that protects cells from oxidative stress (OS) by catalytic neutralization of the toxicity of superoxide radicals via a reaction that converts superoxide ($O_2^-$) to $O_2$ ions and $H_2O_2$.[325–327]

*SOD1* is one of the genes that are most commonly involved in the ALS pathogenesis. *SOD1* mutations have been associated with a great number of familial ALS (fALS) cases, and more than 170 mutations of *SOD1* were found to cause ~20% of the cases of fALS and ~5% of the cases of sporadic ALS (sALS).[328–331] However, it is worth keeping in mind that the numbers reflecting the incidence of *SOD1* mutations in ALS are changing over time, and it is currently accepted that ~10% and ~2% of fALS and sALS cases, respectively, are associated with mutations in the *SOD1* gene.[332] Although many of the SOD1 mutations lead to a loss of function, at least some of the ALS-related mutations are thought to cause the enzyme to gain damaging properties,[333] such as ability to damage mitochondria, proteasomes, and protein chaperones.[334] Conformationally, many SOD1 mutants have similar properties, possessing solvent-exposed hydrophobic regions that increase the propensity of this protein to aggregate.[335]

Interestingly, the "Dr. Jekyll–Mr. Hyde" behavior of SOD1 is not only triggered by mutations, because misfolded WT-SOD1 has also been implicated in sporadic ALS.[335] Curiously, although the discovery of the dominant mutations in the *SOD1* gene made almost 25 years ago[336] marked the beginning of the molecular era of the analysis of ALS, no consensus on the main toxicity of mutant SOD1 has emerged as of yet.[4] Currently, there is a wide spectrum of potential toxic mechanisms proposed to explain how mutations in SOD1 might mediate the degeneration and death of motor neurons,[4] and such a lack of consensus serves as an indication of the overall complexity of this problem.

SOD1 protein maturation involves the appropriate coordination of $Cu^{2+}$ (by His47, His49, His64, and His121) and $Zn^{2+}$ ions (by His64, His72, His81, and Asp84) and the formation of a kinetically stable disulfide bond between Cys57 and Cys146.[337] A very unusual feature of SOD1 is its striking capability to maintain an intrasubunit disulfide bond even under the reducing conditions of the cytosol. In fact, in the vast majority of proteins the sulfhydryl groups of cysteine residues have a pKa >8.0. As a result, in the absence of oxidative stress and in the reducing environment of the cytoplasm at physiological pH, these sulfhydryl groups remain protonated, and cytoplasmic proteins, as a rule, do not contain disulfide bonds.[338,339] The CCS (copper chaperone for superoxide dismutase) is required for the formation of this disulfide bond in SOD1, because the spontaneous disulfide formation in SOD1 by $O_2$ is a slow process, which is greatly accelerated by the $Cu^{2+}$-bound form of CCS.[337] It was pointed out that both metal occupancy and disulfide status are crucial for the formation of a stable functional state of SOD1,[340] because without these factors the protein cannot gain its stable native conformation and cannot dimerize. Obviously, the formation of these functional homodimers with stable native conformation and intact disulfide bonds can also be altered by the pathological SOD1 mutations,[335] or by oxidation, demetallation, and other altered PTMs.[341–344] It was also pointed out that some aberrant PTMs induced by oxidative damage (e.g., appearance of oxidized carbonyl groups in the protein leading to its over-oxidation) force the WT-SOD1 to acquire binding and toxic properties of mutant forms of this protein.[341–344] This gives some support to the idea that SOD1 "is subject to misfolding following the loss of normal PTM and/or the induction of aberrant modification".[335]

There are several ways by which mutated and misfolded SOD1 and misfolded WT-SOD1 might act as a neurotoxin. First, pathogenic SOD1 may exert its neurotoxic effects via glutamate excitotoxicity, which causes an abnormally high release of calcium ions in the post-synaptic cleft that triggers subsequent neurotoxic events including mitochondrial dysfunction. Pathogenic SOD1 is theorized to cause this excitotoxicity due to the loss of SLC1A2/EAAT2/GLT1 (reducing reuptake of the neurotransmitter) and excessive glutamate efflux. Second, astrocytes expressing a SOD1 mutant lack the ability to effectively regulate their $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors, whose subunit composition

is in constant flux, which can cause different permeability. These mutant astrocytes lack the GRIA2/GluR2 subunit of these receptors that increases their permeability to $Ca^{2+}$ ions. This sensitization, along with increased calcium production/decreased reuptake, act in tandem to cause excitotoxicity.[335] Third, OS by reactive oxygen species (ROS) is increased in cells with mutant SOD1. However, this stress is not due to loss of function by SOD1 but rather due to upregulation of the ROS. These ROS are formed via many different mechanisms including NAPDH oxidase and mitochondrial respiration.[335] Fourth, mutant SOD1 puts stress on endoplasmic reticulum (ER) proteins such as DERL1 (derlin 1), and the resulting ER stress has been linked to both familial and sporadic ALS. In particular, DERL1 is crucial for the elimination of misfolded proteins from the endoplasmic reticulum. Interactions between mutant SOD1 and DERL1 cause the inhibition of this process, resulting in ER stress and ultimately apoptosis via ERN1/IRE1 (endoplasmic reticulum to nucleus signaling 1).[335]

The mitochondria is another target for mutant SOD1, which has been shown to accumulate exclusively on the cytosolic side of mitochondria in the spinal cord even before ALS symptoms are observed.[335] Binding of mutant SOD1 to several target proteins, including the integral membrane proteins BCL2 (BCL2, apoptosis regulator) and VDAC1 (voltage dependent anion channel 1), causes irregularities in mitochondrial homeostasis. Over-oxidized WT-SOD1 also forms a toxic complex with BCL2 in the lymphoblasts in some patients with sporadic ALS.[335]

Vesicle transport is extremely important for long motor neurons to function normally, and distortion in this transport can potentially contribute to selective motor neuron death. Therefore, another pathological mechanism of mutant SOD1 has been linked to abnormal axonal synaptic vesicle transport in motor neurons during the early stages of ALS.[345] In fact, at an early asymptomatic ALS stage preceding loss of spinal motor neurons and peripheral axons, fast axonal transport is impaired in transgenic mice carrying G93A $SOD1^{G93A}$ or $SOD1^{G86R}$ mutations[345,346] mutations equivalent to those found in subsets of human fALS. In line with the idea that the oxidized WT-SOD1 shares an aberrant conformation with the ALS-related mutants of this protein, a detailed analysis revealed that recombinant, oxidized WT- SOD1 and the wild-type protein immunopurified from sALS tissues were equally able to inhibit the kinesin-based fast axonal transport, acting similarly to the fALS-linked SOD1 mutants,[343] suggesting an overlapping pathogenic mechanism.[335]

Finally, recent evidence suggests that SOD1 aggregation also exerts toxicity via prion-like propagation. This idea is supported by the fact that aggregating SOD1 is engaged in both cell-to-cell transmission and seeded aggregation. Both wild-type and mutant SOD1 form aggregates that are released from the injured cells as part of an exosome.[347] Also, mutant SOD1 can be released via a main secretory pathway mediated by CHGA (chromogranin A) and CHGB that serve as components of neurosecretory vessels.[348] Likely, this pathway can also be related to the release of the oxidized WT-SOD1 which was shown to interact with CHGB.[341,349] In addition, both wild-type and mutant SOD1 aggregates seed aggregation of WT-SOD1 in vivo, supporting the theory of prion-like propagation of SOD1-related pathology.[335]

#### 4.1.3. SOD1 and biogenesis of stress granules and other PMLOs

It has been established that both the WT-SOD1 and mutant SOD1 forms (mtSOD1 containing A4T, G41S, G85A, or G93A substitutions), as well as TARDBP, and YWHAB/14-3-3 protein $\alpha/\beta$ (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta) can be involved in interaction with *NEFL* (neurofilament light) mRNA and differentially modulate the stability of this mRNA, which is destabilized by mutant SOD1,[350] but stabilized by TARDBP[351] and YWHAB.[352] Furthermore, interaction of WT-SOD1 or mtSOD1 with YWHAB results in efficient disruption of the interaction of YWHAB with *NEFL* mRNA,[353] suggesting that SOD1 might have dual effects on the mRNA stability, via direct binding and by eliminating stabilizing YWHAB-*NEFL* mRNA interaction. Importantly, using RNA-IP-PCR in ALS spinal motor neurons, it has been also demonstrated that *NEFL* mRNA is sequestered in SGs and P-bodies, suggesting that *NEFL* mRNA processing is dramatically altered in ALS.[353] Although the colocalization with the *NEFL* mRNA-containing stress granules and P-bodies was unequivocally established for TARDBP, the ability of YWHAB, WT-SOD1 and mtSOD1 to interact with *NEFL* mRNA suggests that all these proteins can be related to alteration of the processing of this mRNA leading to its compartmentalization within both SGs and P-bodies.[353] It is important to note that the aforementioned *NEFL*, is not the only mRNA sequestered by SOD1 and TARDBP aggregates and SGs. As a matter of fact, it is now well-established that TARDBP and SGs in general bind up thousands of various RNAs, making *NEFL* mRNA, at best, a singular model RNA for the types of cellular mechanisms these promiscuous RNA-binding proteins and RNA granules might be involved with.[354–358]

Direct involvement of SOD1 in the regulation of SG dynamics was recently established by showing the ability of mtSOD1 (L144F) to interact, in an RNA-dependent manner, with G3BP1 that plays a critical role in SG dynamics.[359] In fact, the aforementioned mtSOD1-G3BP1 interaction is rather specific (involving F380 and F382 residues of the RNA-binding RNA-recognition motif [RRM] domain of G3BP1), because almost no interaction is seen between the mtSOD1 and other RNA-binding proteins implicated in ALS.[359] It was also shown that the SG formation induced in N2A cells by hyperosmolar shock and arsenite treatment is delayed by the mtSOD1, indicating that one of the culprits of pathogenic SOD1 mutations in ALS could be related to the alterations of the RNA metabolism and the SG dynamics.[359]

### 4.2. TARDBP/TDP-43 (TAR DNA binding protein)

#### 4.2.1. Structural properties of TARDBP

TARDBP/TDP-43 is an RNA- and DNA-binding protein that is 414 amino acids long with a molecular mass of 44 kDa. TARDBP belongs to the family of the heterogeneous ribonucleoproteins (hnRNPs), members of which are RNA-binding proteins that bind to pre-mRNA and are involved in RNA splicing. The *TARDBP* gene is located on chromosome 1p36.2 and contains 6 transcribed exons.[360] Human TARDBP protein (UniProt ID: Q13148) has an N-terminal domain (NTD,

residues 1–103), 2 RNA recognition motifs, RRM1 (residues 104–200) and RRM2 (residues 191–262), a nuclear export signal (NES), a nuclear localization signal (NLS), and a glycine-rich C-terminal domain (residues 274–413), which is known as a prion-like domain and is predicted to be highly disordered (Fig. 3B). This RRM1-RRM2-C-tail domain organization is similar to that of hnRNP family proteins such as HNRNPA1 and HNRNPA2B1.[361] Although TARDBP is mainly a nuclear protein, it moves between the inside and outside of the nucleus, with this shuttling regulated by its NES and NLS motifs. The C-terminal domain is used by TARDBP for binding to single-stranded DNA, RNA, and proteins, and most of the ALS- and FTLD-related mutations found in TARDBP are located within this prion-like domain encoded by exon 6 of the *TARDBP* gene.[362] This prion-like domain has also been associated with the misfolding and self-aggregation of this protein.[363] According to comprehensive computational analysis, the C-terminal region of human TARDBP contains 4 disorder-based protein-protein interaction sites[201] and, therefore, clearly serves as a major "docking port" for its binding partners.

Human TARDBP is present in 2 proteoforms generated by alternative splicing, with isoform #2 being different from the canonical form of the protein by having a changed N-terminal tail, where the $^{1}$MSEYIRVTEDENDEPIEI$^{18}$ sequence is substituted to MPQMLAGEIWCMLSTIQK, and where the 19–134 region is missing.

#### 4.2.2. Normal and pathological roles of TARDBP

TARDBP has many functions, most of which are related to RNA handling due to its ability to bind RNA through its RNA recognition motifs.[364] Some of these functions include regulation of transcription, pre-mRNA splicing, as well as processing, stability, and transport of mRNA.[365,366] In addition, TARDBP can participate in protein-protein interactions with itself and several other proteins. Examples of its binding partners include HNRNPA1, HNRNPA2B1 and FUS.[367] These proteins are required for TARDBP to perform some of its biological functions.[367] An important feature of TARDBP is its intrinsic ability to form aggregates, and this aggregation process is mediated by the C-terminal fragments of TARDBP.[368] TARDBP is a component of ubiquitin-positive MAPT-negative insoluble protein aggregates in both neurons and glial cells in sporadic ALS.[369] In addition, it was shown that the cleavage of TARDBP by CAPN (calpain) makes this protein more prone for aggregation than the full-length protein, and that both phosphorylated and nonphosphorylated TARDBP fragments resulting from this proteolytic cleavage remain intracellularly long enough to self-aggregate.[370] Furthermore, because the phosphorylated TARDBP becomes resistant to the CAPN cleavage it is likely that phosphorylation (and some other PTMs) may play a regulatory role in the TARDBP pathology of ALS and FTLD.[370] The pathological phosphorylation of TARDBP is controlled by the phosphatase PPP3/calcineurin that can bind to and catalyze the removal of pathological C-terminal phosphorylations of TARDBP.[371]

In line with the hypothesis on the important roles of the TARDBP proteolytic cleavage for pathogenesis of neurodegeneration is the fact that various TARDBP fragments generated by the apoptotic CASP3 (caspase 3) and CASP7, as well as by CASP6 and CASP8 after activation by CASP3, accumulate in motor neurons and serve as a post mortem hallmark of different neurodegenerative diseases.[372] In fact, activation of the CASPs under some stress conditions might result in the fast appearance of the C-terminal fragments (CTFs) of TARDBP with the molecular mass of 35 kDa that accumulate in the cytoplasm, and the formation of this truncated form of TARDBP can be efficiently inhibited by the A90V mutation found in the FTLD/ALS patient with a family history of dementia,[373] whereas another mutant, D169G, is cleaved by CASP3 more efficiently than the wild-type TARDBP.[374] Also, the 35-kDa CTFs of TARDBP form ubiquitin-negative cytoplasmic inclusion bodies, which are mildly toxic, whereas the 25-kDa TARDBP CTFs are able to efficiently form toxic ubiquitin-positive inclusion bodies and sequester cellular RNA.[375] These observations are in line with the hypothesis that the CASP-mediated cleavage pattern of TARDBP determines the rate of clearance and cytotoxicity of this protein.[376] They also indicate that TARDBP possesses dual vulnerability to proteolytic attack by both CAPNs and CASPs.[377]

#### 4.2.3. TARDBP, SGs, and other PMLOs

Undoubtedly, among the ALS-FTLD-related RNA-binding proteins, the involvement of TARDBP in SG formation represents one of the best studied cases of the regulated protein aggregation phenomenon. One of the first reports indicating the existence of a potential link between TARDBP, SGs (which are liquid-like ribonucleoprotein complexes where protein synthesis is temporarily arrested), and ALS was published in 2009, when, based on axotomized motor neuron analysis, it was hypothesized that the upregulated TARDBP expression combined with the prominent localization of this protein to the cytosolic SGs might represent a physiological response to the motor neuron injury caused by degenerative processes, such as ALS.[378] In the same year, careful analysis of the response of a motoneuronal cell line to oxidative stress and environmental insults revealed that TARDBP is capable of efficient SG assembly, with the RRM1 (residues 104–200) and the 216–315 region of the TARDBP C-tail being crucial for the recruitment of this protein into SGs.[379] Also, analysis of the ALS spinal motor neurons revealed that TARDBP plays a fundamental role in compartmentalization of *NEFL* mRNA (processing of which is fundamentally altered in ALS) within SGs and P-bodies.[359] The SG recruitment of TARDBP was further evidenced by the large scale analysis of the TARDBP interacting proteins, many of which are known SG components.[364] Furthermore, this analysis revealed that the TARDBP interacting proteins can be functionally clustered into the cytoplasmic/translation- and nuclear/splicing-related PPI networks, suggesting that TARDBP can function in both the nucleus and the cytoplasm, playing multiple roles in RNA metabolism.[364]

Many subsequent studies provided direct support to the idea that TARDBP, SGs, and ALS-FTLD are intimately linked. For example, an analysis of the cultured cells under the stress conditions revealed the existence of the TARDBP-SG association promoted by both the direct binding of TARDBP to specific SG proteins, such as TIA1, and via the direct interactions of TARDBP with RNA.[380] Furthermore, it was pointed out that the "regulated protein aggregation" leading to the SG formation

might be associated with the ALS pathology, because the increased formation of TARDBP-containing SGs is associated with accumulation of TARDBP-based detergent insoluble complexes both in cell cultures and pathological brain tissues.[380] Also, treatment with the translational inhibitors that suppress or reverse SG formation inhibits the formation of inclusions mediated by the wild-type or mutant TARDBP,[380] suggesting that there is an interplay between TARDBP aggregation, SG formation, and the ALS-FTLD-associated TARDBP mutations, an understanding of which is vital to the clarification of the roles of TARDBP in neurodegeneration.[294] It was indicated that pathological TARDBP aggregates can be formed via at least 2 different mechanisms, either following the "independent model", where the formation of such aggregates is independent of SG formation, or via the "precursor model," where SG formation might seed the formation of TARDBP aggregates.[294] Therefore, TARDBP undergoes transitions between soluble, droplet, and aggregate phases, and these transitions might have important implications for the pathological aggregation and disease development.[381]

There are also several studies emphasizing that the TARDBP-based formation of SGs, due to being regulated by various means, represents an illustrative example of "regulated protein aggregation". For example, TARDBP involvement in SG formation in SH-SY5Y neuronal-like cells exposed to chronic oxidative stress induced by overnight treatment with the mitochondrial inhibitor paraquat is affected by inhibition of MAPK8/JNK1, MAPK3/ERK1 or MAPK14/p38.[382] In addition to oxidative stress, formation of TARDBP-positive SGs can be promoted by ER stress, which is induced in both familial and sporadic forms of ALS, and the extent of which is dependent on the overexpression of ALS-linked mutant TARDBP.[383] Intracellular levels of 4-hydroxynonenal (HNE, a marker of oxidative stress), are elevated in sporadic ALS patients. Treatment of cells with HNE induces noticeable aggregation and phosphorylation of TARDBP, affects cellular localization of this protein, and decreases its accumulation in SGs, suggesting that the aberrations in the SG dynamics induced by the enhanced HNE levels might represent an ALS risk factor.[384]

The paraquat-induced formation of the TARDBP-positive SGs in SH-SY5Y cells is successfully suppressed by inhibiting the MAPK/ERK-induced phosphorylation of TARDBP by bioavailable *bis*(thiosemicarbazonato)copper(II) complexes.[385] Subsequent screening of various kinase inhibitors using an in vitro model of the formation of TARDBP-positve SGs revealed that the TARDBP accumulation is successfully decreased by the inhibitors of CDKs (cyclin-dependent kinases) and GSK3 (glycogen synthase kinase 3), and these same inhibitors are able to reverse the pre-formed TARDBP-positive SGs.[386] A more detailed analysis revealed that the SG integrity is dependent on CDK2 phosphorylation.[387] Here, only phosphorylated CDK2 is able to colocalize with TARDBP accumulated within SGs and to phosphorylate HNRNPK (heterogeneous nuclear ribonucleoprotein K) that interacts with TARDBP, whereas nonphosphorylated CDK2 fails to phosphorylate HNRNPK, preventing its incorporation into SGs and thereby preventing accumulation of TARDBP in SGs.[387]

The fact that the ALS-linked mutations of TARDBP (A315T and Q343R) are able to increase the tendency of this protein not only to aggregate, but also to form SGs provides another important link between ALS pathology and aberrant SG dynamics.[388] Fig. 7 provides an illustration for this idea by presenting effects of pathological TARDBP mutants, such as the fALS-associated mutation A315T, sALS-related mutations G294A and N390S, the fALS- and sALS-associated mutation G348C, and truncated TARDBP (residues 1–324) on SG formation and characteristics in HEK293T cells.[389] Fig. 7 shows that although the wild-type and all mutant proteins are expressed at similar levels (Fig. 7B) and are predominantly localized to the nucleus in the unstressed HEK293T cells (see "Control" line in Fig. 7A), a multitude of SGs is formed in the cytoplasm of stressed HEK293T cells by each pathological mutant after one h of osmotic stress induced by 0.4 M sorbitol, and these "pathological" SGs are more numerous and noticeably larger than the SGs formed by the wild-type TARDBP under the same conditions (see Fig. 7C for the quantification of the size of SGs formed by various TARDBP species).[389] However, not all ALS-related mutations in TARDBP are made equal, and a recent study showed that the A382T mutation causes a significant reduction in the number of SGs per cell, reduces the percentage of cells that form SGs, and leads to a significant decrease of viability, suggesting that the A382T mutation-induced loss of TARDBP function in SG nucleation reduces the ability of cells to respond to stress.[390]

Although many of the aforementioned findings seem to link TARDBP, SGs, and ALS pathogenesis, since alterations in SG dynamics have been suggested to play a key role in the TARDBP protein aggregation process,[283,292,294,299,391,392] this hypothesis remains hotly contested. This is because SGs are typically observed in studies that are usually conducted under gross overexpression of TARDBP or other ALS proteins, or in the presence of the nonpathologically relevant mutations to those proteins, or following harsh treatments with agents inducing cell stress, such as sodium arsenite or $H_2O_2$; SGs are not too often observed in ALS patients. To date, the primary criticism of the SG hypothesis is that it generated few "leads" that have withstood the test of time, proven highly reproducible in other laboratories, or proven more generally true in ALS patients. Despite these concerns, the important roles of TARDBP in regulation of SG biogenesis and dynamics are not under question in model studies when cells are stressed and undergo development of SG (and other PMLOs). Obviously, future studies are needed to shed more light on this important problem.

### 4.3. FUS/fused in sarcoma/translocated in liposarcoma (FUS RNA binding protein)

#### 4.3.1. Structural properties of FUS

The second most common suspect linking aberrant SGs to ALS-FTLD is FUS (FUS RNA binding protein). In fact, FUS is an important player in ALS, mutations of which are responsible for a noticeable subset of both fALS and sALS,[393,394] where they account for ~4% and 1% of total cases, respectively.[360,395] Furthermore, FUS mutations account for ~35% of fALS in patients younger than 40 years old.[396] The *FUS* gene is positioned within chromosome 16 (its cytogenic location is 16p11.2, which is the short [p] arm of chromosome 16 at position 11.2) and has

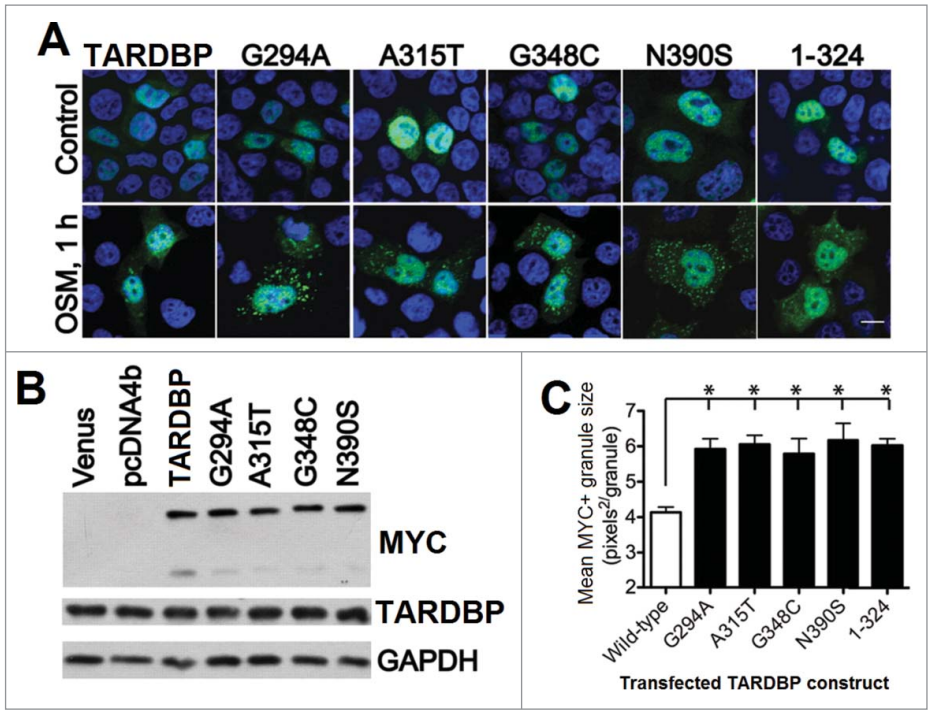


**Figure 7.** Effect of the ALS-related mutations within the C-terminal region of human TARDBP on the generation and morphology of SGs in response to osmotic stress. (A) Localization of the exogenous wild-type TARDBP, as well as transiently expressed pathological TARDBP mutants and TARDBP lacking the C-terminal tail in HEK293T cells. Exogenous TARDBP was stained with anti-MYC antibody; nuclei, with ToPro-3. Bar: 10 $\mu$m. (B) Levels of expression of proteins shown in panel (A). (C) Quantification of SG size (pixels$^2$/granule) following 1 h of sorbitol stress. Shown are mean granule sizes $\pm$ SEM for wild-type (open bar) and mutant (filled bars) TARDBP ($^*$, $P < 0.05$). Reproduced with permission from ref. 389.

15 exons. It encodes a 526 residue-long RBP, which belongs to the FET family of RPBs that includes FUS, EWSR1, and TAF15 (TATA-box binding protein associated factor 15).[397,398] Human FUS (UniProt ID: P35637) contains several distinct functional domains including a RNA-recognition motif and a highly-conserved C-terminal NLS.[395] This C-terminal domain is a hot-spot for the identified ALS-related mutations.[399]

### 4.3.2. Biological and pathological roles of FUS

The FUS protein is expressed both in the nucleus and cytoplasm, and can shuttle between these 2 locations. Although the nucleus of neurons is a site of the predominant localization of FUS in the norm, characteristic FUS-immunoreactive inclusions are found in the cytoplasm of the ALS–FUS and FTLD–FUS patients, suggesting that the mislocalization of this protein to the cytoplasm might contribute to the neurodegeneration via the gain-of-toxicity mechanism.[399,400] This protein is involved in several biological functions, which include DNA repair,[401,402] regulation and control of transcription of target genes,[403] and RNA processing via regulation of pre-mRNA splicing.[354] Recent studies revealed that target gene expression can be significantly altered by FUS mutations due to the ability of mutant forms to bind to the target gene mRNA.[404] It was also indicated that the ALS-associated R521C mutation in human FUS is able to interfere with the normal function of the wild-type protein by interacting with it and by interfering with normal FUS binding to HDAC1 (histone deacetylase 1).[405] This mutant FUS also forms more stable complexes with the mRNA of BDNF (brain derived neurotrophic factor) than the wild-type protein and altered alternative splicing of this mRNA.[405] Furthermore, an interesting feed-forward regulatory loop has been established, where some mutant forms of FUS protein upregulate microRNAs *MIR141* and *MIR200A* that, in their turn, affect FUS protein synthesis.[406] Although both TARDBP and FUS share the ability to form abnormal cytoplasmic aggregates in tissues of ALS and FTLD patients via interaction with mRNAs, these proteins bind different sets of cytoplasmic mRNAs that, however, converge on common cellular pathways, and differently affect the post-transcriptional fate of their partner mRNAs in the cytoplasm.[407] Similar to TARDBP, FUS can interact with RNA and with several members of the hnRNP family of RNA-binding proteins.[408] Although aggregation of FUS and TARDBP is observed in the cytoplasm of ALS patients, the related mechanisms of pathogenesis are assumed to be different.[370] This hypothesis is based on the observations that TARDBP aggregates are observed primarily in adult patients, whereas FUS aggregates are found in juvenile patients.[370]

### 4.3.3. FUS and PMLOs

In ALS-FTLD, the cytoplasm-located SGs preferentially contain mutant FUS but not the wild-type protein,[409,410] suggesting that the pathogenic FUS mutations are related to the altered dynamics of SG assembly and reflect the presence of important differences between normal and disease physiology.[409–411] Furthermore, OS causes mutant FUS recruitment into SGs, leading to the sequestering of the wild-type FUS, disruption of RNA processing, and initiation of cell death.[412] The involvement of fALS-associated mutations in FUS cytoplasmic mislocalization is attributed to the fact that the majority of these

mutations affect the NLS positioned within the C terminus of FUS, thereby impairing nuclear import of this protein.[400,413,414] Similar effects were described for the protein with a deleted C-terminal 32 amino acid residues containing an effective NLS.[415]

All these observations emphasize the crucial roles of the ALS mutations or deletion of the NLS of FUS in the distortion of RNA metabolism via impairment of FUS nuclear localization and induction of the cytoplasmic inclusions and SGs.[415] The biological and pathological importance of the observed effects of the C-terminally truncated FUS was recently reemphasized by finding an ALS-related mutation in the *FUS* gene consisting of a 2-base pair deletion, *c.1509_1510delAG*, resulting in a truncated protein, p.G504Wfs*12, lacking the NLS.[416] Expression of this truncated FUS leads to the severe cytoplasmic mislocalization of mutant FUS and strong colocalization of this protein with SGs.[416]

ALS-related mutations in FUS have a complex effect on SG dynamics. In fact, although SG assembly is delayed in cells expressing mutant FUS, once formed, the mutant-FUS-containing SGs are noticeably different from the SGs lacking FUS, being more dynamic, larger, and more abundant, and disassembling more rapidly once stress is removed.[417] ALS-linked C-terminal mutations have profound effects on cellular localization and functions of FUS protein, which is diffusely mislocalized in the cytoplasm in the form of SGs and spontaneously formed large aggregates. In addition to mutant FUS, these inclusions contain wild-type FUS and RNA-binding proteins HNRNPA1, HNRNPA2B1, and SMN1.[418] These large, spontaneously formed FUS mutant-derived aggregates in the cytoplasm sequester a variety of RNA binding proteins and mRNAs suggesting toxic gain of function leading to the disruption of the various aspects of RNA equilibrium and biogenesis.[418]

In addition to ALS-related mutations, the methylation of FUS arginines by PRMT1 (protein arginine methyltransferase 1) plays an important role in regulation of the nuclear-cytoplasmic shuttling of wild-type FUS and its amyotrophic lateral sclerosis 6-associated mutants participating in the mutation-induced toxic gain of function.[419,420] Also, oxidative stress promotes the accumulation of mutant FUS in cytoplasmic SGs leading to the sequestration of the wild-type protein.[421]

Curiously, the abilities of the endogenous wild-type FUS to translocate from the nucleus to the cytoplasm and to be engaged in SG formation are related to the physiological cellular response to the hyperosmolar stress induced by sorbitol, with the cell viability in response to sorbitol being mostly lost in cells with reduced expression of FUS.[422] It was pointed out that the recruitment of FUS in SGs might represent a unique cellular defense mechanism against irreversible aggregation of FUS protein mislocalized to the cytoplasm.[423]

### 4.4. Protein C9orf72 and dipeptide repeat proteins Poly (GA), Poly(GR), Poly(GP), Poly(PA), and Poly(PR) generated due to the hexanucleotide repeat expansion of the intronic noncoding region of the C9orf72

#### 4.4.1. Structural properties of C9orf72 and DPRs

The most common genetic change underlying both FTLD and ALS is related to the alterations of the *C9orf72* (chromosome 9 open reading frame 72) gene. The cytogenic location of *C9orf72* is 9p21.2, which is the short (p) arm of chromosome 9 at position 21.2. The *C9orf72* gene consists of 12 exons, and multiple transcript variants encoding different isoforms can be generated by alternative splicing. The major pathological alteration of *C9orf72* is the hexanucleotide repeat expansion (GGGGCC/CCCCGG) in the noncoding region located in an intronic region of the *C9orf72* gene between its noncoding exons 1a and 1b.[424,425] This hexanucleotide repeat expansion can vary from 10 to thousands of repeats and is associated with 30 to 60% of fALS cases, 8% of sALS cases, 25% of FTLD cases, and up to 88% of cases in familial ALS-FTLD.[332,424–430] Said hexanucleotide repeat expansion is now considered as the most common cause of familial and sporadic TARDBP-positive FTLD and ALS (C9orf72-FTLD/ALS).[431,432]

Although the hexanucleotide repeat expansion affects the noncoding region of the *C9orf72* gene, the expanded repeats can be bi-directionally transcribed, generating numerous sense and antisense repeat RNAs.[433–436] Furthermore, the resulting sense and antisense repeat RNAs can be translated to generate a series of the dipeptide repeat proteins (DPRs or C9-related polypeptides synthesized via RAN-translation). When sense GGGGCC repeat RNAs are used as mRNAs in the RAN-translation, 3 different proteins, poly(GA), poly(GR), and poly(GP), are synthesized,[437,438] whereas RAN-translation of the antisense CCCCGG repeat RNAs generates poly(PA), poly(PR), and poly (GP) proteins.[434,436,439] Expanded repeat-containing RNAs are engaged in the formation of RNA foci that sequester RNA-binding proteins, whereas RAN-generated DPRs are found as aggregates throughout the CNS of C9orf72-ALS-FTLD patients.[434,436–440]

Alternative splicing of human *C9orf72* generates 2 proteoforms (UniProt ID: Q96LT7), a canonical or long isoform consisting of 481 residues, and a short isoform that has 222 residues due to lack of the C-terminal half of the sequence (residues 223–481).

As was already mentioned, due to their highly redundant sequences, all DPRs, including poly(GA), poly(GR), and poly (GP), poly(PA), and poly(PR),[433-436] synthesized by RAN-translation using the sense and antisense repeat RNAs generated as a result of the bidirectional transcription of the hexanucleotide repeat expansions of the *C9orf72* gene are expected to be mostly disordered, and in agreement with this expectations, all these DPRs are also predicted to be highly disordered.[201]

#### 4.4.2. C9orf72 in the norm and pathology

Although the biological functions of the C9orf72 protein remained unknown for a long time, recent analysis revealed that this protein can be involved in interactions with CFL1 (cofilin 1) and other actin binding proteins, thereby regulating the axonal actin dynamics and also serving as a modulator of small GTPases.[441] Also, C9orf72 takes part in the regulation of endosomal trafficking.[442] Furthermore, in neuronal cell lines, primary cortical neurons and human spinal cord motor neurons, C9orf72 colocalizes with RAB/RAS-related proteins implicated in autophagy and endocytic transport—RAB1, RAB5, RAB7 and RAB11—indicating that C9orf72 might be related to autophagy regulation.[442]

The repeat-positive patients are characterized by the reduced expression levels of both AS isoforms of human C9orf72.[443–445] However, the exact position of the C9orf72 loss-of-function mechanism in the C9-FTLD/ALS pathogenesis is not clear as of yet. In fact, although several studies ruled out a loss of function of the C9orf72 protein as a contributor to ALS pathology[446] (because, e.g., cellular survival in vitro[447] or in vivo[448] of both cortical and motor neurons is not affected by the reduction of the C9orf72 levels), many laboratories continue to study the roles of C9orf72 in ALS pathology. Also, C9orf72-depleted motor neurons are characterized by reduced axonal actin dynamics, which is associated with the altered capacity of neurons to maintain axons and axon terminals in the cellular pathology underlying ALS and FTLD.[441] The reduction in the axonal actin dynamics is associated with a pathway involving phosphorylation of the actin binding protein CFL1 through the C9orf72-dependent modulation of the activity of the small GTPases ARF6 (ADP ribosylation factor 6) and RACK1 (receptor for activated C kinase 1) leading to the enhanced activity of LIMK1 LIM domain kinase 1) and LIMK2.[441] Furthermore, loss of C9orf72 in mouse brain neuroblastoma cells alters functions of a complex containing WDR41 (WD repeat domain 41) and SMCR8/Smith-Magenis syndrome chromosome region, candidate 8) that is involved in modulation of the GTPase functions of the members of RAS oncogene family, RAB8A and RAB39B, resulting in impaired autophagy in neurons.[449]

It was pointed out that the mutations in C9orf72 not only cause the appearance of malformed RNA molecules, but also promote mislocalization of TARDBP to the cytoplasm.[450] Conversely, in various models, DPRs alter cellular functions and induce toxicity in different ways.[440] For example, the arginine-rich DPRs—poly(GR) and poly (PR)—display the highest toxicity in primary neurons and fly models, where they trigger nucleolar stress, nuclear transport defects, protein mislocalization, and RNA processing alterations.[447,451,452] In contrast, expression of poly(GA) proteins in primary neurons results in aggregation of the transport factor UNC119, proteasome impairment, and impairment of RAD23B/HR23B and nucleocytoplasmic transport proteins.[453–455] Finally, very little or almost no toxicity is reported for poly(GP) and poly(PA).[434,447]

#### 4.4.3. C9orf72, DPRs, and SGs

It has been established that the expanded repeats located within the noncoding region of the *C9orf72* gene can be bidirectionally transcribed, and both sense and antisense repeat RNAs are engaged in the formation of RNA foci in the CNS of ALS and FTLD patients.[433–436] Besides RNA foci containing hexanucleotide sense and antisense repeat RNAs, the afflicted patients are characterized by the presence of diverse proteinaceous inclusions throughout the central nervous system (CNS).[456] These inclusions are composed of the DPRs (or C9-related polypeptides synthesized via RAN-translation) generated from the GGGGCC repeat RNA via repeat-associated non-ATG (RAN) translation. Therefore, the hexanucleotide repeat expansion of the *C9orf72* gene produces a double-hit impact on the cells via sequestration of some of the RNA-binding proteins into RNA foci (e.g., as is observed when a $GGGGCC_{31}$ repeat expression in NSC34 and HeLa cells causes sequestration of PURA and its binding partner, FMR1/FMRP, in the intracytosolic SGs)[457] and via formation of various intracellular inclusions (including SGs) containing RAN-generated DPRs.[458] Systematic analysis of the interactomes of all DPRs revealed that arginine-containing poly(GR) and poly(PR) are engaged in binding to RBPs and proteins with low complexity sequence domains, which are common components of several PMLOs, such as SGs, nucleoli, and the nuclear pore complex.[458] Furthermore, poly(GR) and poly(PR) alter dynamics and functions of multiple PMLOs.[458] For example, localization of these 2 DPRs to the nucleolus induces suppression of ribosomal RNA synthesis, impairs SG formation, and causes translocation of the key nucleolar component NPM1 (nucleophosmin 1), leading to nucleolar stress and cell death.[459] Finally, the C9orf72 protein itself is able to interact with several ALS-related proteins, such as UBQLN2, HNRNPA2B1, HNRNPA1, and ACTB (actin beta).[442] Furthermore, in cells overexpressing *C9orf72*, in addition to the formation of nuclear C9orf72 aggregates, prominent HNRNPA1- and HNRNPA2B1-positive SGs emerge, and the proportion of these HNRNPA1- and HNRNPA2B1-positive SGs noticeably increases when the cells overexpressing *C9orf72* are treated with proteasome inhibitors.[442]

### 4.5. PFN1 (profilin 1)

#### 4.5.1. Structural properties of PFN1

Human PFN1 (profilin 1; UniProt ID: P07737) is a small, globular, actin-binding protein, mutations in which constitute a relatively rare cause of fALS,[460] mostly among patients with European ancestry.[461] The *PFN1* gene is characterized by the cytogenetic location 17p13.2, which is the short (p) arm of chromosome 17 at position 13.2. It has 6 exons and can generate at least 6 alternatively spliced variants.

#### 4.5.2. Normal and pathological functions of PFN1

Human PFN1 is a 140 residue-long ubiquitous eukaryotic protein that serves as a major regulator of filamentous actin growth via binding actin-ADP monomers, promoting the conversion of actin-ADP to actin-ATP, transporting the actin-ATP monomers, and interactions within FMNs/formins located at the growing end of actin filaments.[462,463] PFN1 also interacts with phosphoinositides, thereby serving as a link between the phosphatidylinositol cycle and actin polymerization.[464,465] Besides serving as an essential component of the cytoskeletal rearrangement signaling pathway, PFN1 can interact with a large cohort of proteins with poly-l-proline stretches.[466,467] Curiously, only mutant forms of PFN1 are engaged in the formation of insoluble aggregates, disrupt cytoskeletal structure, inhibit filamentous actin formation, and elevate UBC (ubiquitin C) and SQSTM1/p62 (sequestosome 1) levels in motor neurons, whereas the wild-type protein does not have such activities.[468]

It was shown recently that the native conformation of PFN1 in vitro is severely destabilized by the ALS-linked mutations, and the turnover of the PFN1 mutant forms is dramatically accelerated in cells.[469] This mutation-induced structural destabilization of PFN1 defines the increased propensity of ALS-linked variants to aggregate and also their loss-of-function phenotypes.[469] In SH-SY5Y neuroblastoma cells, transient expression of ALS-linked PFN1 mutants (and not of the

wild-type protein) causes the formation of cytoplasmic SQSTM1- and ubiquitin-positive aggregates that sequester endogenous TARDBP, induce accumulation of TARDBP, and promote conversion of normal TARDBP into its abnormal (detergent-insoluble and phosphorylated) form, suggesting the presence of the gain-of-toxic function scenario, where the ALS-related mutations equip PFN1 with a novel toxic function that causes TARDBP aggregation.[470]

#### 4.5.3. PFN1 and stress granules

Although mutations in the *PFN1* gene are associated with fALS and provide a potential link between the actin cytoskeleton abnormalities and ALS pathogenesis, these ALS-linked *PFN1* mutations are rare, and the actual role of mutations in this protein in neurodegeneration is poorly understood. Recently, some insights into the potential pathological mechanisms of the *PFN1* mutations were gained using the budding yeast *Saccharomyces cerevisiae* that has a *PFN1* ortholog (in fact, *PFN1* is highly conserved through evolution) as a model and utilizing the power of yeast genetics in the form of an unbiased, genome-wide synthetic lethal screen with yeast cells lacking profilin (*pfy1Δ*).[471] This analysis revealed that in the *pfy1Δ* background, deletion of some genes related to SGs and P-bodies is synthetically lethal, suggesting that PFN1 can be related to the SG and P-body dynamics.[471] This hypothesis is supported by the analysis of mouse primary cortical neurons and human cell lines that show association of PFN1 and the related protein PFN2 with the SGs, and revealed that SG dynamics is indeed altered by the ALS-linked mutations in PFN1.[471]

### 4.6. Nucleoporin GLE1 (GLE1, RNA export mediator)

#### 4.6.1. Structural properties of GLE1

The gene encoding the human nucleoporin GLE1 protein is located at position 34.11 of the long (q) arm of chromosome 9 (cytogenetic location: 9q34.11) and contains 22 exons, generating at least 11 transcript variants encoding different alternatively spliced isoforms. GLE1 serves as a shuttling mRNA export factor that is needed for the export of the poly(A) tail-containing mRNAs from the nucleus into the cytoplasm,[207-210] and also may play a role in the terminal step of mRNA transport through the nuclear pore complex (NPC) via interaction with nucleoporins NUPL2/CG1 (nucleoporin like 2) and NUP155.[472] Being an essential multifunctional modulator of DEAD-box RNA helicases in yeast, Gle1 is involved in translation initiation through regulation of a DEAD-box protein, the initiation factor Ded1,[473] and plays a role in termination of translation via interaction with another DEAD-box protein, Dbp5.[474] There are 2 alternative splicing-generated isoforms of GLE1 in human, with the canonical form (or GLE1B) consisting of 698 residues, and with the GLE1A form missing the C-terminal tail (residues 660–698) and harboring the change IEAI → YQAC at residues 656–659 constituting a new C terminus. Although GLE1B and GLE1A share 655 identical residues, these isoforms are characterized by different intracellular distribution. In fact, although the canonical GLE1B isoform is localized predominantly to the NPC, the short GLE1A isoform is spread diffusely throughout the nucleus and cytoplasm.[208] Localization of GLE1B to the NPC is ensured by the interaction of its N-terminal tail (residues 1–29) with NUP155,[212] as well as via binding of the C-terminal tail (residues 656–698) to NUPL2.[472] In addition to its NUP155- and NUPL2-binding motif located at the N- and C-terminal tails, respectively, GLE1 (UniProt ID: Q53GS7) possesses a region related to the mediation of protein shuttling between the nucleus and the cytoplasm (residues 444–483). This protein also has 2 coiled-coil regions (residues 151–277 and 306–356).

#### 4.6.2. Functions and dysfunction of GLE1

Two rare deleterious ALS-associated mutations in GLE1 (a splice site 1965-2A>C and a nonsense 209C>A mutation) deplete GLE1 at the nuclear pore, thereby abrogating function of this protein in nuclear mRNA export.[475] The splice site mutation affects intron 14 of GLE1, where it destroys a splice acceptor site, resulting in the expression of a mutant protein, in which the C-terminal 44 residue-long region is replaced with a novel 88 amino acid C-terminal domain.[475] A nonsense c.209C>A mutation introduces a premature stop codon in exon 2 of *GLE1* thereby encoding a truncated GLE1-S70X protein of only 69 residues.[475] The resulting c.209C>A mRNA is highly unstable and the truncated GLE1-S70X protein is not found.[475] In addition to these 2 deleterious mutations, some ALS patients possess a missense variation c.2089C>T encoding a mutant protein harboring the R697C substitution.[475] Functional implementations of this mutation have not been assessed as of yet.

Functional analysis revealed that the splice site mutant possesses functions ascribed to both GLE1A and GLE1B isoforms, suggesting that it is not subject to the normal mechanisms of GLE1 regulation.[476] Besides causing ALS, mutations in human GLE1 are associated with autosomal recessive conditions that lead to death of fetuses before the 32$^{nd}$ week of gestation or soon after birth, lethal congenital contracture syndrome 1 (LCCS1 caused by the T144 → TPFQ and R569H mutations) and lethal arthrogryposis with anterior horn cell disease (LAAHD caused by the V617M and I684T mutations).[477–479]

#### 4.6.3. GLE1 in PMLOs

One of the fALS forms associated with mutations in the *GLE1* gene result in the production of a protein that is defective in SG regulation, and has some translation-related cytoplasmic functions.[480] As was already pointed out, GLE1 protein is present in cells as at least 2 isoforms generated by alternative splicing. These 2 forms are differently expressed (with the canonical form being more common), have different cellular localizations (with GLE1B being primarily localized to the nuclear envelope rim and with GLE1A being mostly found in the cytoplasm), and have different functions (with GLE1B interacting with NPC and playing a role in efficient and expedient mRNA export from the nucleus,[481] and with GLE1A being involved in essential cytoplasmic functions related to translational modulation and regulation of proper assembly and disassembly of SGs[480]).[208,210,212,472] The effect of the ALS-related mutation on the GLE1 sequence can be considered from the GLE1A and GLE1B angles. From the GLE1B perspective, this mutation causes the replacement of the 44-amino acid NUPL2 binding site with a novel 88 amino acid C-terminal domain, whereas from the GLE1A perspective, the *GLE1-c.1965-2A>C* mutation

in the *GLE1* gene results in the replacement of a short C-tail with a long C-terminal domain. Because this new inserted domain cannot bind to the nucleoporin NUPL2,[475] it was expected that GLE1-IVS14-2A>C would be functionally similar to GLE1A.[476] In agreement with this hypothesis, GLE1-IVS14-2A>C is absent from the nuclear envelope rim, causes an increase in the cytoplasmic functions of GLE1, is recruited to SGs, and is able to regulate assembly and disassembly of SG2, resulting in the formation of larger SGs.[476] Furthermore, both the GLE1A and the GLE1-IVS14-2A>C form cytoplasmic "fibrous" aggregates that do not colocalize with the components of SGs but recruit TARDBP and the molecular chaperone HSP90.[476] However, in contrast to normal GLE1A that is not involved in mRNA export from the nucleus, GLE1-IVS14-2A>C is able to support some level of mRNA export in cells with endogenous GLE1 depletion.[476] Therefore, the ALS-related GLE1-IVS14-2A>C isoform is capable of acting as both GLE1A and GLE1B, and may contribute to ALS pathogenesis via distortion of the normal mechanisms of GLE1 regulation.[476]

### 4.7. Transcriptional activator PURA/Pur-α (purine rich element binding protein A)

#### 4.7.1. Structural properties of PURA

The gene encoding the transcriptional activator PURA/PUR1 (purine rich element binding protein A) has the cytogenetic location 5q31.3 (i.e., at position 31.3 of the long [q] arm of chromosome 5) and contains 5 exons. Originally, transactivator PURA was shown to bind to the purine-rich single strand of the PUR element located at a putative origin of replication site in the *MYC* gene.[482] Subsequent studies revealed that PURA is involved in (mostly negative) regulation of several genes, such as *SPN/leukosialin/CD43* (sialophorin),[483] *FAS* (Fas cell surface death receptor),[484] *APBB1/FE65* (amyloid beta precursor protein binding family B member 1),[485] *MBP* (myelin basic protein),[486] *CHRNB4* (cholinergic receptor nicotinic beta 4 subunit),[487,488] and *ACTA2* (actin, alpha 2, smooth muscle, aorta).[489]

Human PURA (UniProt ID: Q00577) is a 322-residue long protein that possesses 2 regions with compositional biases, a Gly-rich domain (residues 11–53) and a Gln- and Glu-rich region (residues 293–322) that might serve as a part of the transcriptional activation domain, and a central DNA-binding domain containing 3 class I repeats and 2 class II repeats.[490]

#### 4.7.2. Biological and pathological roles of PURA

PURA is a highly conserved, ubiquitous and multifunctional protein engaged in a sequence-specific interaction with single-stranded DNA and RNA.[220] Among different functions ascribed to this protein are targeting mRNA to neuronal dendrites,[491–493] DNA replication, DNA repair, and gene transcription.[490,494,495] Being a single-stranded nucleic acid-binding protein, PURA possesses DNA helix-destabilizing helix unwinding activities.[496] The preferred DNA sequence recognized by PURA is composed of the GGN repeats, and binding of PURA to this recognition sequence is accompanied by the formation of multimeric complexes and is controlled and amended by interaction with other transcription factors.[490,497,498] In addition to interaction with single-stranded DNA and RNA, PURA can bind to a cohort of cellular regulatory proteins, such as the CCNA1 (cyclin A1)-CDK2 complex,[499,500] CCNT1 (cyclin T1),[501] CDK9,[501] E2F1 (E2F transcription factor 1),[502] RB1 (RB transcriptional corepressor 1),[503] SP1 (Sp1 transcription factor),[486] and YBX1/YB1 (Y-box binding protein 1).[504] Furthermore, this protein is considered to be a major player in the regulation of the cell cycle and can be involved in oncogenic transformation,[490] and its intracellular level varies during the cell cycle, peaking during mitosis and decreasing at the onset of S phase.[499]

It was pointed out that PURA might be involved in the pathogenesis of the FUS mutation-associated form of ALS through the control of mRNA translation, indicating that altered protein synthesis may be implicated in the disease.[505]

#### 4.7.3. Roles of PURA in SG biogenesis

Engagement of PURA in ALS pathogenesis is related to the sequestration of this important RNA/DNA-binding protein in the inclusions containing the expanded GGGGCC repeat RNAs generated by the expansion of the hexanucleotide repeats in the first intron of *C9orf72*.[506–508] Also, in the presence of the $GGGGCC_{31}$ repeats, PURA efficiently accumulates in the intracytosolic granules positively stained with SG markers.[457]

Furthermore, PURA is able to specifically bind to the FUS C-terminal region, physically interact with the mutated FUS in an RNA-dependent manner, and colocalize with the mutated FUS to the SGs found in the motoneuronal cells derived from ALS patients.[505] In ALS patient cells carrying disease-causing FUS mutations and challenged with stress and in mammalian neuronal cells, PURA efficiently colocalizes with mutant FUS in constitutive SGs.[509] Because SG formation in mammalian cells is significantly reduced by the shRNA-mediated knockdown of endogenous PURA, it has been concluded that this protein is essential for SG assembly.[509]

### 4.8. FLCN (folliculin)

#### 4.8.1. Structural properties of FLCN

Human *FLCN/BHD* with cytogenetic location 17p11.2, which is the short (p) arm of chromosome 17 at position 11.2, contains 32 exons, and encodes a 579 residue-long protein (folliculin). FLCN serves as a tumor suppressor, mutations in which are associated with BHD syndrome, a rare inherited genodermatosis characterized by kidney tumors, lung cysts, and benign tumors of the hair follicle (hair follicle hamartomas).[510–513] Mutations in FLCN are also associated with primary spontaneous pneumothorax[514–516] and familial renal cell carcinoma.[517] FLCN is highly conserved across species, with human protein showing 92% identity to the mouse FLCN, being 22–36% identical (44–56% similar) to the *Drosophila melanogaster* protein and 27–28% identical (44–52% similar) to the *Caenorhabditis elegans* ortholog.[511]

Human FLCN (UniProt ID: Q8NFG4) has 1 coiled-coil region (residues 287–310) and might be present in 3 isoforms generated by alternative splicing, where isoforms #2 and #3 differ from the canonical form by missing long regions C-terminally located regions (residues 343–579 and 198–579) and possessing the DLEEESESWD…SGCGSWQPRK→GEAGVLL PGP…LREAHPPISV and CPGREGPIFF…FLLGKVRGII→SLVATEPVSV…

ELREESCWTC changes at residues 291–342 and 134–197, respectively. Curiously, most cancer-related mutations in this protein are associated with the C-terminal truncations in FLCN suggesting that this domain might play a role in tumor suppression.[518] A crystal structure solved for the C-terminal domain of human FLCN (residues 341–566) shows that this domain is characterized by an $\alpha\beta$ structure, where a core $\beta$-sheet and helices packed on the one side are followed by an all-helical region, and which is similar to the differentially expressed in normal cells and neoplasia (DENN) domain of DENND1B/connecdenn 2 (DENN domain containing protein 1B; PDB ID: 3V42, see ref. 518).

#### 4.8.2. Functionality and dysfunctionality of FLCN

FLCN is related to energy and/or nutrient sensing and the MTOR (mechanistic target of rapamycin) pathway via the FLCN-interacting proteins FNIP1 and FNIP2 that also bind to AMP-activated protein kinase (AMPK), which is involved in energy sensing and negative regulation of MTOR activity.[519,520]

FLCN is related to ALS via its direct interactions with TARDBP (where the residues 202–299 of FLCN interact with the RRM domains of TARDBP) leading to the modulation of TARDBP cytoplasmic translocation and aggregation.[521] FLCN is needed for TARDBP nuclear export, but not nuclear import.[521] The overexpression of FLCN is associated with enhanced TARDBP cytoplasmic mislocalization, whereas reduced FLCN levels lead to the preferential deposition of TARDBP to the nucleus.[521]

#### 4.8.3. FLCN and PMLOs

The FLCN-driven cytoplasmic mislocalization of TARDBP leads to SG formation and accumulation of TARDBP aggregates that are recognized by the UPS and autophagy-lysosome pathway.[521] Curiously, under stress conditions, FLCN is needed for the successful incorporation of TARDBP into the SGs, because in the arsenite-treated cells containing endogenous FLCN, TARDBP is preferentially found in the cytoplasm where it colocalizes with SGs, whereas after arsenite treatment of the FLCN-depleted cells, TARDBP dissociates from the SGs and shuttles back into the nucleus.[521]

### 4.9. RBM45 (RNA binding motif protein 45)

#### 4.9.1. Structural properties of RBM45

The gene encoding human RBM45/DRB1 (RNA binding motif protein 45) has the cytogenic location 2q31.2. The *RBM45* gene has 8 exons and can generate 3 alternatively spliced isoforms.

The amino acid sequence of human RBM45 (UniProt ID: Q8IUH3) consists of 476 residues and possesses 3 RRMs (residues 26–106, 121–195, and 392–464) as well as a C-terminal nuclear localization sequence (within the C-tail residues 454–472).[522] There is also a homo-oligomer assembly (HOA) domain located within the linker region between RRM2 and RRM3 that promotes homo-oligomerization of RBM45 and is highly conserved across species.[522] In addition to the full-length canonical form, alternative splicing generates 2 proteoforms, where intron retention in isoform 2 generates a sequence missing the 225–226 dipeptide, missing a C-terminal domain (residues 331–476) and having a D330E substitution. Isoform 3 is minimally different for the canonical form, being characterized by the missing 225–226 dipeptide.

#### 4.9.2. Biology and pathology of RBM45

RBM45 protein belongs to the family of neural RRM-type RNA-binding proteins that play a number of important roles in neural development.[523] It was also reported that human RBM45 preferentially interacts with the poly(C) RNA.[523] Although this protein is predominantly found in the nucleus, it contains a nuclear localization signal and can shuttle between the nucleus and cytoplasm.[524] Recent immunoprecipitation and mass spectrometry analysis revealed that 132 proteins can specifically interact with RBM45, and that this excessive protein-protein interaction (PPI) network includes many RBPs engaged with RBM45 mostly via RNA-dependent interactions in the nucleus.[525] Among the RBM45-interacting proteins discovered in this study were several ALS-linked RBPs, such as FUS, HNRNPA1, and TARDBP,[525] and among the biological processes and pathways ascribed to the RBM45-interacting proteins are RNA processing, RNA splicing, cytoplasmic RNA translation, and EIF2 (eukaryotic translation initiation factor 2) and EIF4 pathways.[525]

Recently, it was reported that levels of RBM45 are statistically elevated in the cerebrospinal fluid of patients with sporadic and familial ALS, and cytoplasmic inclusion bodies containing RBM45, ubiquitin, and TARDBP are found in 91% of ALS, 100% of FTLD–TARDBP and 75% of Alzheimer disease cases.[526] Immunochemical analysis also revealed that RBM45 forms a punctate pattern within nuclei of neurons and glia in the brain and spinal cord that lack either TARDBP or ubiquitin. Patients with the *C9orf72* hexanucleotide repeat expansion display the most extensive RBM45 pathology,[526] likely due to the aforementioned binding preference for poly (C) RNA. However, the generality of this conclusion is questionable, because only 3 *C9orf72* patients were analyzed in this study. Subsequent studies also showed that RBM45 is able to bind and colocalize with the C-terminal fragment of TARDBP.[527] This protein also modulates the antioxidant response in ALS through interactions with KEAP1 (kelch like ECH associated protein 1), which is an inhibitor of the antioxidant response transcription factor NFE2L2 (nuclear factor, erythroid 2 like 2).[528] It was also shown that one of the pathological mechanisms linking RBM45 with ALS can be related to the mislocalization of this protein and perturbation of its nuclear-cytoplasmic trafficking that could be responsible for the induction of the formation and accumulation of toxic cytoplasmic aggregates.[524]

#### 4.9.3. RBM45 and SGs

The cytoplasmic inclusions containing RNA-binding protein RBM45, being present in neurons and glia in a majority of ALS and FTLD patients, incorporate TARDBP and are marked with ubiquitin.[526] It was pointed out that, while similar to TARDBP and FUS, RBM45 possesses RRMs, and does not have a characteristic glycine-rich prion-like domain (PrLD),[526] but contains a bipartite nuclear-localization sequence located at the C-terminal region.[522] Besides RNA binding, RBM45 is able to interact with the C-terminal domain of TARDBP[527] both in the nucleus and cytoplasm,[522] can bind to FUS in an RNA-dependent

manner,[522] and is able to form homo-oligomers via the evolutionarily conserved motif located within the linker region between RRM2 and RRM3.[522]

Although RBM45, TARDBP and FUS are predominantly localized to the nucleus in healthy cells, these proteins form cytoplasmic inclusions in cells of ALS-FTLD patients, with RBM45 homo-oligomerization being needed for the efficient association with other ALS-linked proteins.[522] Also, when cells are exposed to oxidative stress, all 3 proteins shuttle from the nucleus to the cytoplasm, where they are engaged in SG formation.[283,299,528,529] Altogether, currently available data suggest that RBM45 self-oligomerization in the cytoplasm plays a role in the incorporation of this protein into SGs, where it promotes RBM45 interaction with TARDBP.[522]

### 4.10. SS18L1/CREST (SS18L1, nBAF chromatin remodeling complex subunit)

#### 4.10.1. Structural properties of SS18L1

The *SS18L1/CREST/KIAA0693* (SS18L1, nBAF chromatin remodeling complex subunit) gene is located on chromosome 20q13.33 and contains 11 exons, encoding a protein required for calcium-dependent dendritic growth and branching in cortical neurons.[530]

Human SS18L1 (UniProt ID: O75177) is a 396-residue-long protein that has an N-terminal autoinhibitory-autoregulatory domain (residues 1–148) needed for SMARCA4/BRG1 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4) binding,[530] followed by a methionine-rich domain (residues 149–232), a multifunctional domain (MFD, residues 246–317) related to the regulation of the subcellular localization and transactivation of SS18L1 and involved in the protein self-dimerization,[531] and a C-terminal transactivation domain (residues 325–396) related to the control of nuclear localization of SS18L1 (residues 334–396) containing a binding region crucial for interaction with CREBBP (residues 387–396).[530] Furthermore, there are 4 potential SH2-binding motifs in human SS18L1, residues 50–53, 353–356, 371–3789, and 391–394. The C-terminal half of SS18L1 (residues 190–396) is characterized by low sequence complexity (it possesses compositional bias, due to being enriched in Gln residues).[530] It was also pointed out that this C-terminal part satisfies the criteria for a PrLD,[530,532] serving as a primary cause of SS18L1 aggregation, a statement supported by the observation that protein aggregation is completely abolished by the deletion of this domain.[533]

Human SS18L1is present as at least 5 isoforms generated by alternative splicing, where, in comparison with the full-length canonical form, isoforms 2, 3, and 4 are characterized by missing N-terminal regions 1–23, 1–86, and 1–131, respectively, and isoform 5 possesses the MSVAFA SARP…SKGKTAECTQ → MQSLSTEARY…ICPRSPPARR substitution at the 1–49 region.

#### 4.10.2. Normal and pathological functions of SS18L1

SS18L1 is another protein whose mutations are associated with ALS.[532,534] SS18L1 is a nuclear calcium-regulated protein involved in transcriptional activation via its interaction with CREBBP[535] mediated by the last 9 residues of SS18L1, and in chromatin remodeling via binding to chromatin remodeling proteins ARID1A/BAF250 (AT-rich interaction domain 1A) and SMARCA4/BRG1.[530] In fact, SS18L1 serves as a constituent of the chromatin remodeling complex including SMARCD3/BAF (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3) playing an important regulatory role at various stages of neural development by modulating transcription of specific sets of genes.[536] Because SS18L1-deficient mice display defective dendritic branching, early lethality, and motor disturbances, it has been hypothesized that the physiological function of this protein is related to the control of the normal development of neuronal dendritic trees.[530]

Association of SS18L1 with ALS pathology is based on finding mutated forms of this protein (e.g., Q388stop and Ile123-Met) in sporadic ALS,[532] and Ala264Thr and *p. Gln222_Ser224del* mutations in patients with the familial form of ALS.[534] Besides being involved in ALS, SS18L1 is associated with synovial sarcoma, which is an aggressive soft tissue tumor caused by the t(X;18)(p11;q11) translocation, where the *SS18* gene on chromosome 18 is fused to one of the *SSX* genes on the X chromosome.[537-540] Among such fusions are *SS18-SSX1, SS18-SSX2*, and *SS18-SSX4* that account for more than 95% of the synovial sarcomas[537-540] and a *SS18L1-SSX1* fusion generated by the in-frame fusion of nucleotide 1216 (exon 10) of *SS18L1* with nucleotide 422 (exon 6) of *SSX1*.[541] In the resulting chimeric SS18L1-SSX1 protein, the last 8 residues of SS18L1 are replaced by 78 C-terminal residues of the SSX1 protein, which is a transcriptional repressor domain.[541]

#### 4.10.3. SS18L1 and biogenesis of stress granules and other PMLOs

In the nucleus, SS18L1 is localized to the nuclear bodies of unknown nature that are positive for the histone acetyltransferase CREBBP/CBP, and the C-terminal 164 residues are needed for this nuclear body localization of SS18L1.[542] Furthermore, SS18L1 belongs to the set of paraspeckle proteins.[543] A recent study showed that SS18L1 is involved in interaction with FUS and possesses a strong tendency for aggregation.[533] In its aggregated form, SS18L1 affects morphology and integrity of paraspeckles via trapping other paraspeckle proteins,[533] thereby supporting a hypothesis that compromised paraspeckle formation can serve as a pathogenic factor in ALS-FTLD.[544,545] Finally, similar to many other ALS-FTLD-related proteins, SS18L1 is found in SGs.[533] Therefore, SS18L1 possesses an intricate ability to be recruited to at least 3 different types of PMLOs, such as CREBBP-containing nuclear bodies, paraspeckles, and SGs.

SS18L1 is one of the ALS-related proteins that contain a PrLD. Curiously, a recent comprehensive review indicated that almost 70 human RBPs possesses PrLDs, which are "low-complexity domains that possess a similar amino acid composition to prion domains in yeast, which enable several proteins, including yeast Sup35 and Rnq1, to form infectious conformers, termed prions."[546] Human RBPs routinely utilize PrLDs in their numerous functions. Furthermore, the presence of PrLDs defines the ability of RBPs to undergo LLPTs leading to the formation of various PMLOs.[546]

### 4.11. HNRNPA1 (heterogeneous nuclear ribonucleoprotein A1) and HNRNPA2B1

#### 4.11.1. Structural properties of HNRNPA1 and HNRNPA2B1

Human genes *HNRNPA1* (with cytogenetic location 12q13.13, which is the long [q] arm of chromosome 12 at position 13.13) and *HNRNPA2B1* (located on chromosome 7p15.2, which is the short [p] arm of chromosome 7 at position 15.2) encode members of a family of ubiquitously expressed hnRNPs. The *HNRNPA1* and *HNRNPA2B1* genes have 13 and 15 exons, respectively, and are able to generate multiple spliced isoforms (at least 9 and 13, respectively).

Both HNRNPA1 and HNRNPA2B1 have similar domain organization. Human HNRNPA1 (UniProt ID: P09651) is 372-residue-long protein possessing 2 globular domains (A and B, residues 4–94 and 95–108) portions of which form RRMs (residues 14–97 and 105–184). Region 218–240 represents the RNA-binding RGG-box, whereas residues 320–357 constitute the nuclear targeting sequence (M9). The C-terminal part of HNRNPA1 (residues 195–372) is a Gly-rich PrLD. There are 2 alternatively spliced isoforms of this protein, where the HNRNPA1-B isoform is considered as a canonical full-length form of the protein, and the HNRNPA1-A isoform (which is 20 times more abundant than HNRNPA1-B) is considered different from the canonical form due to missing residues 252–303.[547-549] Analogously, human HNRNPA2B1 (UniProt ID: P22626) is a 353-residue-long protein that has 2 RRMs (residues 21–104 and 112–191), a low complexity, Gly-rich C-terminally located PrLD (residues 193–253), a nuclear targeting sequence (residues 308–347), and a nuclear localization signal (residues 9–15). Structural information is available for the N-terminal regions of both proteins (residues 2–196 of human HNRNPA1 containing 2 RNA recognition motifs, PDB ID: 2LYV[550]; and residues 1–103 corresponding to the first RRM of the human HNRNPA2B1, PDB ID: 1X4B).

Both HNRNPA1 and HNRNPA2B1 proteins are present as 2 proteoforms generated by alternative splicing, with the HNRNPA2B1 proteoforms being different by a 12 amino acid insertion close to the N terminus,[547-549] but both containing C-terminally located PrLDs that encompass residues 197–353 in HNRNPA2B1 and residues 185–341 in hnRNPA2.[363,551] The HNRNPA1 proteoforms are different due to missing amino acids 252–303 in the shorter HNRNPA1-A isoform, but both harbor PrLDs located at their C-termini that span residues 186–320 in HNRNPA1-A and residues 186–372 in HNRNPA1-B.[363,551]

#### 4.11.2. Biological and pathological functions of HNRNPA1 and HNRNPA2B1

HNRNPA1 and HNRNPA2B1 are 2 ubiquitous RNA-binding proteins with a PrLD that are mutated in ALS.[552] HNRNPA1 is an RNP involved in several RNA metabolic processes, such as pre-mRNA splicing and transport of cellular RNAs, which it accomplishes by shuttling between the nucleus and the cytoplasm.[361,553-555] Curiously, different RNA-binding profiles were assigned to the cytoplasmic and nuclear HNRNPA1, with the cytoplasmic protein possessing a high affinity for the AU-rich elements,[556,557] and with the nuclear protein having a high affinity for a polypyrimidine stretch bordered by AG at the 3′ ends of introns.[558,559]

HNRNPA2B1 is among the most abundant RBPs in eukaryotic cells and is highly expressed in brain and muscle. The major function of this RBP is to form the core of the RNP complex that associates with the nascent pre-mRNAs, packaging them into hnRNP particles and thereby playing important roles in various processes ranging from transcription, to pre-mRNA processing, to RNA nuclear export and subcellular location, to mRNA translation, and to modulating the stability of mature mRNAs.[560] Human HNRNPA2B1 is engaged in the formation of a multitude of HNRNP particles via interaction with several (at least 20) other HNRNPs and heterogeneous nuclear RNA.[560] The name to this protein is given due to the existence of 2 functional isoforms, HNRNPA2 and HNRNPB1, generated by alternative splicing, with the HNRNPB1 serving as a full-length canonical form and with the HNRNPA2 isoform missing residues 3–14 close to the N terminus.

Mutations in PrLD of human HNRNPA2B1 (Asp302Val) and HNRNPA1 (Asp314Val or Asp314Asn) are associated with ALS and multisystem proteinopathy (also known as inclusion body myopathy with early-onset Paget disease and FTLD). To gain some knowledge on the molecular mechanisms connecting mutations in HNRNPA1 and HNRNPA2B1 with fALS, a structure-based threading algorithm ZipperDB that is designed to predict the presence in a query protein of steric zippers, short (6-amino acid-long) segments able to form 2 self-complementary $\beta$-strands,[561] was recently used.[552] This analysis revealed that the potent steric zipper motifs were introduced by various ALS-associated mutations into the PrLDs of HNRNPA1 and HNRNPA2B1.[552] A subsequent study provided experimental support to this hypothesis and revealed that although the wild type versions of HNRNPA1 and HNRNPA2B1 have a noticeable intrinsic tendency to form self-seeding fibrils, this fibrillation propensity is dramatically enhanced by the disease-associated mutations that accelerate the formation of self-seeding fibrils that cross-seed polymerization of wild-type proteins.[551]

#### 4.11.3. HNRNPA1, HNRNPA2B1, and PMLOs

In addition to enhancing the formation of cytoplasmic inclusions, the ALS-related mutations foster the excess incorporation of HNRNPA2B1 and HNRNPA1 into SGs.[551] In fact, the constitutive SGs contain higher levels of mutant forms of the HNRNPA2B1 and HNRNPA1 proteins than the corresponding wild-type proteins. Furthermore, arsenite treatment of model cells results in more rapid incorporation of mutant proteins into SGs than the wild-type proteins, indicating that disease-related mutations alter the recruitment of HNRNPA2B1 and HNRNPA1 not only into the cytoplasmic inclusions but also into SGs.[551]

### 4.12. ATXN2 (ataxin 2)

#### 4.12.1. Structural properties of ATXN2

Human ATXN2 is a 1,313-residue-long protein involved in the trafficking of EGFR (epidermal growth factor receptor) via binding to SH3GL2 (SH3 domain containing GRB2 like 2, endophilin A1) and SH3GL3 (SH3 domain containing GRB2 like 3, endophilin A3), which are the brain-expressed members of the SH3GL/endophilin A family involved in synaptic vesicle

endocytosis.[562] ATXN2 is a 1,313 residue-long protein most known for its involvement in the pathogenesis of spinocerebellar ataxia type 2 (SCA2) caused by the expansion of the polyglutamine (polyQ) region of this protein encoded by the expanded CAG trinucleotide repeat within the open reading frame of the *ATXN2* gene located at position 24.12 on the long (q) arm of chromosome 12 (cytogenetic location: 12q24.12) and encoding multiple transcript variants due to alternative splicing and the presence of 26 exons. In fact, although CAG repeats are known to vary in length in normal alleles of *ATXN2*, the most common allele carries 22 CAG repeats and the maximal *ATXN2* repeat size is 31, whereas expansion of the *ATXN2* CAG repeat region to carry 34–59 repeats is associated with SCA2.[563-568]

In addition to the canonical full-length form, human ATXN2 (UniProt ID: Q99700) has at least four alternatively spliced isoforms. Isoform 2 is missing the C-terminal tail (residues 996–1313) and harbors the PLYPIPMTPMPVNQAK → YQICPNSGKTSIIRVP substitution in the preceding region (residues 980–995). Isoform 3 is the shortest and the most diversified form of ATXN2, missing N-terminal (residues 1–981) and C-terminal tails (residues 1258–1313), missing the 1106–1123 region, and possessing YPIPMTPMPVNQAKTYR→MYYAVEILFNRQSAFFS and AHVQSGMVP→VIPALAN FL substitutions at residues 982–998 and 1249–1257, respectively, in addition to harboring the Ile1124Val substitution. In isoform 4, the C-tail (residues 1244–1313) is missing, whereas isoform 5 is characterized by missing regions 1–265 and 1106–1124 and possesses the ACPKLPYNKETSPSF YFAI → V substitution. The canonical form has several regions with compositional bias, such as three Pro-rich regions (residues 47–158, 551–734, and 929–1085), a poly-Pro region (residues 55–64), a poly-Gln region (residues 166–187), and a poly-Ser region (residues 213–223). It was indicated that several of these isoforms are present in mice and humans, might have different functions, and are expressed in different tissues, such as brain, spinal cord, cerebellum, heart, and placenta.[569]

#### 4.12.2. Normal and pathological roles of ATXN2

This cytoplasmic RNA-binding protein is considered as an ALS susceptibility factor, because intermediate-length polyglutamine expansions (Q27-Q33) in ATXN2 are associated with increased ALS risk.[570-572] Curiously, although SCA2 is associated with the polyQ expansions exceeding 34 repeats all encoded by pure CAG repeat expansions in the *ATXN2* gene, the corresponding expansions associated with ALS are all interrupted, containing 1 to 3 CAA codons (also encoding glutamine) within the CAG repeats.[573] Despite the fact that CAG expansion in genes encoding several proteins such as ATXN1, ATXN2, ATXN3, ATXN7, TBP (TATA-box-binding protein), ATN1 (atrophin 1), and HTT (huntingtin) is associated with a set of polyQ diseases, expansions in polyQ disease genes other than ATXN2 are not associated with ALS, suggesting that the repeat-induced alterations in the biological functions of ATXN2 and not the mere presence of an expanded polyQ repeat represent the ALS risk factor.[574] Because no *ATXN2*-associated polyglutamine expansion was found in patients with sporadic and familial FTLD, it has been concluded that this polymorphism is ALS specific.[575] The pathological effect of the ATXN2 polyQ expansion in ALS is associated with the enhancement of the stress-induced activation of CASP3 and increase TARDBP pathological modifications, such as C-terminal cleavage and phosphorylation.[576] Furthermore, the presence of the ATXN2 intermediate-length expansion affects the morphology of the TARDBP inclusions, because the motor neurons of ALS patients harboring ATXN2 polyQ expansions contain primarily skein-like or filamentous TARDBP deposits and do not contain large round Lewy body-like inclusions abundantly found in ALS patients without ATXN2 polyQ expansions.[577]

#### 4.12.3. ATXN2 in the biogenesis of PMLOs

In addition to TARDBP and FUS, ALS-FTLD-related mutations, which are known contributing factors to SG formation due to the mislocalization of carrier proteins to the cytoplasm, ATXN2 is also known as a constituent protein of SGs in mammalian cells, suggesting the existence of the common pathological cascade formed by TARDBP, FUS, and ATXN2 and related to the aberrations in SG dynamics.[578] The importance of ATXN2 in the controlling dynamics of PMLOs is supported by the observations that this protein can interact with the DEAD/H-box RNA helicase DDX6 (DEAD-box protein 6), which is an important component of P-bodies and SGs, and that the alterations in the ATXN2 levels might interfere with the assembly of SGs and P-bodies.[579]

Based on the analysis of cellular ALS models, it was also suggested that ATXN2 with an ALS-linked intermediate-length repeat (ATXN2 Q31) might serve as a potent modifier of the FUS-based pathology, promoting FUS translocation to the cytoplasm, inducing ER stress, and promoting Golgi fragmentation.[580] Furthermore, because ATXN2 colocalizes with FUS in motor neurons derived from sALS and FUS-linked fALS patients, colocalizes with FUS in the ER-Golgi compartments in neuronal cell lines and coprecipitates with FUS in ALS spinal cord lysates, it was hypothesized that the interplay between the mutated FUS and ATXN2 with intermediate-length polyQ expansions provides potential molecular and cellular mechanisms linked to ALS pathology.[580] Similarly, a connection between the TARDBP-based pathology and ATXN2 with intermediate-length polyQ expansions was noted based on the comparative analysis of the TARDBP-positive inclusions in the ALS cases with and without ATXN2 possessing intermediate-length polyQ expansions.[577] Here, fewer motor neurons in the ALS cases without extended ATXN2 contain abundant large round (likely SGs) and skein-like TARDBP inclusions, whereas only skein-like TARDBP inclusions are predominantly found in almost ALS cases harboring ATXN2 polyQ expansions.[577] It was also found that an increased ATXN2 expression might cause TARDBP and FUS mislocalization, leading to RNA dysregulation.[581,582]

### 4.13. TIA1 (TIA1 cytotoxic granule associated RNA binding protein)

#### 4.13.1. Structural properties of TIA1

In humans, the *TIA1* gene is located on chromosome 2p13.3 (at position 13.3 of the short [p] arm of chromosome 2). It has 15 exons, generates at least 11 transcript variants and the gene

product is an RNA-binding protein involved in alternative pre-RNA splicing and regulation of mRNA translation.[583] TIA1 is another RNP containing poly-glycine-rich PrLD that promotes reversible physiological aggregation of TIA1 into SGs,[584] but, under stress conditions, can initiate formation of pathological RNA granules.[585,586] TIA1 is characterized by a well-developed interactome, shown to co-immunoprecipitate with 163 brain proteins.[586] Therefore, based on the presence of a domain with profound intrinsic aggregation propensity and the overall high binding promiscuity of TIA1 it is not surprising to find that abnormal TIA1-containing deposits are found in the brain tissue of patients with AD, ALS, Creutzfeld-Jakob disease, frontotemporal dementia with Parkinsonism (FTDP-17), FTLD–TDP43, Huntington disease, and spinal muscular atrophy.[290,584,585]

Human TIA1 (UniProt ID: P31483) is a 386-residue-long protein that contains 3 RRMs (residues 7–83, 106–184 and 214–286), which serve to bind RNA and a Gly-rich domain that mediates protein aggregation.[290] In addition to the canonical full-length proteoform, 2 isoforms are produced by alternative splicing. Here, isoform 2 has a SSTVVSTQRSQD → N substitution at the 93–104 region, whereas isoform 3 is missing the C-terminal tail (residues 215–386) and has a SNTKQLSY-DEVVNQSSPSNC → CRCIGEEKEMW NFGEKYARF substitution in the preceding region (residues 195–214).

#### 4.13.2. Biology and pathology of TIA1

TIA1 can serve as an apoptosis-promoting factor that is able to modulate alternative splicing of dedicated transcripts.[587] Careful analysis of the consequences of the *Tia1* deletion in mouse spinal cord and cerebellum revealed several strong dysregulations, including noticeable effects on the cell cycle and apoptosis regulators, as well as on lipid storage and membrane trafficking factors.[588]

#### 4.13.3. TIA1 and SGs

TIA1 is thought to serve as one of the core RBPs responsible for the primary step (nucleation) of RNA granule formation followed by recruitment of secondary RBPs to form mature RNA granules, such as SGs. SGs act as key players in environmental stress- and viral infection-induced translational suppression due to their role in deciding the fate of sequestered mRNA by sorting it for re-initiation, storage, or degradation.[586] One of the facts supporting the involvement of TIA1 in SG biogenesis is formation of SGs in cells infected with poliovirus.[589] Such SGs, formed at early stages of infection, contain TIA1, translation initiation factors, RNA binding proteins, and mRNA.[589] TIA1 shuttling from the nucleus in response to environmental stress, and subsequent aggregation of a C-terminal proteolytic TIA1 fragment containing glutamine-rich PrLD play a crucial role in the formation of cytoplasmic SGs,[269,277,590,591] suggesting that the controlled prion-like aggregation of TIA1 might be related to the regulation of SG formation.[290,592] In fact, it was pointed out that TIA1 is one of a few key RBPs, such as TIAL1/TIAR, ZFP36, G3BP1, G3BP2, and FMR1, which play a crucial role in the initial stress-induced nucleation of SGs.[269,290,592] The central role of TIA1 and TIAL1 in SG biogenesis and related stress-induced inhibition of translation initiation is supported by the fact the stress-induced phosphorylation of translation initiation factor EIF2A is followed by the TIA1- and TIAL1-dependent recruitment of most cytoplasmic mRNAs to SGs.[277] In this way, TIA1 and TIAL1 act as translational silencers needed for controlling the duration of stress-induced translational arrest.[277]

### 4.14. MAPT (microtubule associated protein tau)

#### 4.14.1. Structural properties of MAPT

The *MAPT* gene located on chromosome 17q21.31 (at position 21.31 of the long [q] arm of chromosome 17) has 16 exons and the gene product is a cytosolic axonal protein that binds and stabilizes microtubules,[593,594] thereby serving as an important structural component of the microtubule-based transport system of the neurons.

Human MAPT (PMID: P10636) exists as a family of proteoforms migrating in SDS gel electrophoresis at close bands of 55–62 kDa. Alternative splicing of *MAPT* mRNA is (at least in part) responsible for this heterogeneity. In fact, although the *MAPT* gene has 16 exons, only 8 of them (1, 4, 5, 7, 9, 11, 12, and 13) are found in all isoforms of this protein and are therefore considered as constitutive, whereas the remaining exons (2, 3, 4A, 6, 8, 10, and 14) are subject to alternative splicing.[595] Because exons 1 and 14 are parts of the promoter and the 3′ untranslated region of the *MAPT* mRNA, respectively, they are not found in any isoform of *MAPT*. None of the brain-specific *MAPT* isoforms have exons 4A, 6, and 8, which are found only in the *MAPT* mRNAs of the peripheral tissue.[596,597] The variability of the brain-specific *MAPT* isoforms is caused by the alternative splicing of exons 2, 3, and 10 (see ref. 597) that generates 6 mRNA isoforms[598,599] containing 2+3+10+, 2+3+10−, 2+3−10+, 2+3−10−, 2−3−10+, and 2−3−10− combinations of these exons and encoding 6 protein isoforms. Because exons 2 and 3 each encode 29-residue-long sequences located in the N-terminal part of MAPT (designated N inserts), and because exon 10 encodes 1 of the 4 31-residue-long microtubule-binding repeats located within the C-terminal part of MAPT and encoded by exons 9, 10, 11, and 12 (designated as R regions), alternative splicing generates MAPT variants that differ from each other by having either 0, 1, or 2 N-terminal inserts, and 3 or 4 C-terminally located R-regions (designated, respectively, as 2N4R, 2N3R, 1N4R, 1N3R, 0N4R, and 0N3R). The longest isoform in the CNS (441 amino acids total) has 4 repeats (R1, R2, R3, and R4) and 2 inserts, whereas the shortest isoform (352 amino acids total) has 3 repeats (R1, R3, and R4) and no insert.[600,601] Although the normal cortex is characterized by equal expression levels of the 3- and 4-repeat forms, the ratio of isoforms is changed in tauopathies.[602,603] Furthermore, in the peripheral nervous system and almost in all CNS neurons that extend processes into the peripheral nervous system, the big MAPT (with a molecular mass of ∼110 kDa) is expressed that contains an additional 254-residue insert in the amino-terminal half encoded by the additional large exon (4a) included in the corresponding mRNA.[604-606]

Phosphorylation and other PTMs serve as an additional source of MAPT microheterogeneity.[607] GSK3B, CDK5, and MAPK kinases are involved in phosphorylation of many residues of MAPT during brain development[608] and multiple sites of MAPT are phosphorylated by several kinases in vitro (for a

review, see ref. 609). The primary targets of the in vitro phosphorylation are residues within the microtubule-interacting region (repeat-containing domain) and its flanking regions. Many of these sites are also phosphorylated in paired helical filaments (which are the major form of MAPT deposition in AD).[610,611] In fact, 10 major phosphorylation sites have been identified in MAPT isolated from paired helical filaments from patients with AD.[610,611]

#### 4.14.2. MAPT function and dysfunction

Among functions ascribed to MAPT in vitro are binding to microtubules, regulation of their assembly, and control of their dynamic instability.[612-616] In situ, the axons have high levels of MAPT,[617] and brain cells in general are estimated to have high proportion of this protein, which might account for 0.025–0.25% of total soluble brain protein.[618,619] Although at normal conditions, the levels of the microtubule-unbound MAPT are kept low, the increase of soluble MAPT caused by the impairment of the protein-degradation system[620] or aberrant phosphorylation[621] might result in aggregation of this protein.[622]

Aggregation of MAPT is associated with a broad spectrum of neurodegenerative diseases collectively known as tauopathies (e.g., AD, FTLD, ALS, progressive supranuclear palsy, cortico basal degeneration, Pick disease, etc.).[623] FTLD is a group of complex diseases characterized by high heterogeneity of their pathological features. In fact, pathologically, FTLD variants are diversified and classified based on the major specific protein found in their inclusions, with FTLD–MAPT (which is an FTLD variant where MAPT serves as the major specific protein of the inclusions) being one of the most common pathological subtypes of this disease.[624]

Among the mechanisms connecting MAPT to ALS is cleavage of this protein and generation of the neurotoxic $MAPT_{45–230}$ fragment that is highly abundant in lumbar and cervical spinal cord specimens obtained from ALS subjects, being present in the ALS upper motor neurons located in the precentral gyrus, and forming aggregates in the spinal cord of ALS patients.[625]

#### 4.14.3. MAPT stress granules, and other PMLOs

ALS and FTLD are tauopathies, which are a large group of neurodegenerative diseases associated with aggregation and dysfunction of MAPT. Curiously, the TIA1 protein, which is typically considered as a SG marker and participates in SG nucleation, was recently shown to be localized to the AD-related neurofibrillary tangles composed of the hyperphosphorylated and aggregated MAPT, with TIA1 being able to directly interact with MAPT.[585] Although normal cytosolic MAPT only weakly interacts with TIA1, the abnormal hyperphosphorylation promotes this interaction.[585] A dedicated study was recently conducted to check if, in its turn, MAPT can be associated with TIA1-containing SGs.[626] This study revealed that when MAPT-expressing cells are treated with arsenite, a prominent SG response is observed and some formed SGs contain MAPT.[626] Furthermore, when extracellular MAPT is internalized in the recipient cells (in line with the cell-to-cell propagation of the MAPT-related pathology model[627]), a prominent formation of SGs is observed, and MAPT is abundantly present in these cytoplasmic nonvesicular cellular bodies.[626] Because in these studies, a pseudohyperphosphorylated MAPT-E14 variant carrying 14 phosphomimetic (serine/threonine to glutamate) mutations is more efficient in induction of SG formation and is able to more strongly interact with TIA1, it has been hypothesized that the hyperphosphorylation-induced oligomerization may be an important factor promoting SG recruitment of the internalized MAPT species.[626] Based on these observations it was suggested that SGs and TIA1 might be important players in the cell-to-cell transmission of MAPT pathology.[626]

## 5. ALS- and FTLD-related proteins, PMLOs, and autophagy

In order to appropriately balance sources of energy at critical times in development and in response to nutrient stress, a cell can utilize a self-degradative process known as autophagy (self-eating) that is responsible for the disposal of various intracellular species.[103,628-634] Macroautophagy (hereafter autophagy) provides a way for elimination of aberrant protein aggregates, superfluous or damaged organelles, and even entire bacteria.[635] Structures doomed for autophagy-based clearance are first sequestered by phagophores, the precursors to autophagosomes, which are large, double-membrane bound vesicles, and then delivered into the interior of the lysosome or vacuole, where they are digested by resident hydrolases.[635,636] When the autophagosome fuses with the lysosome, the autolysosome is formed, and the content of the autophagosome is catabolized.[637,638] It is important to remember that the formation of a new autophagosome does not cover all autophagy processes.[298] For example, in a chaperone-mediated autophagy, individual protein substrates are directly targeted to the lysosome by chaperones and then translocated across the lysosomal membrane, whereas in microautophagy, invagination of the lysosomal membrane directly engulfs the cytoplasmic content.[639] Because autophagy can sequester and degrade large protein complexes and entire organelles, it represents an important alternative to the ubiquitin-proteasome system.[298,638,640]

In ALS, aberrant SGs cannot disassemble and therefore accumulate and are consequently degraded by autophagy.[641] There are several observations linking together ALS- and FTLD-related proteins, stress granules (and potentially other PMLOs), and autophagy. Furthermore, it is thought that there might be a special chaperone-mediated SG surveillance system, granulostasis, which might regulate composition and dynamics of SGs, thereby playing a role in ALS pathogenesis.[641,642] The first lines of evidence on the connection between autophagy and ALS have been derived from animal models.[298] For example, an autophagy pathway was shown to be enhanced in a transgenic mouse model of SOD1-ALS,[643] and also was observed in spinal cord tissues from SOD1-ALS patients.[644] In line with the granulostasis hypothesis,[642] recruitment of chaperones prevents formation of aberrant SGs in cells expressing ALS-linked variants of SOD1, and promotes disassembly of such SGs when the stress subsides.[645]

TARDBP can also be involved in autophagy regulation, mostly by controlling biogenesis of autophagosomes and lysosomes.[646-649] For example, autophagosome-lysosome fusion is

blocked by TARDBP loss of function.[648] Also, TARDBP targets a key component of the MTORC1 complex, RPTOR/raptor (regulatory associated protein of MTOR complex 1), thereby regulating the MTORC1-TFEB (transcription factor EB) signaling pathway.[649] Stability of *RPTOR* mRNA is decreased when TARDBP function is lost, leading to the enhancement of autophagosomal and lysosomal biogenesis in an MTORC1-dependent manner. Furthermore, loss of TARDBP is also associated with impaired autophagosome-lysosome fusion in an MTORC1-independent manner.[649]

Another study in this area was a work by Farg et al., who established that besides regulating endosomal trafficking, C9orf72, via its interaction with RAB proteins related to autophagy and endocytic transport, such as RAB1, RAB5, RAB7 and RAB11, can be involved in regulation of autophagy in neurons.[442] This idea is further supported by the colocalization of C9orf72 with UBQLN2 and autophagosome marker MAP1LC3B/LC3B (microtubule associated protein 1 light chain 3 beta)-positive vesicles, and comigration of this protein with lysosome-stained vesicles in neuronal cells.[442] Finally, a possible dysregulation of trafficking in ALS patients bearing the C9orf72 repeat expansion was postulated based on the immunohistochemical analysis of motor neurons of ALS patients that revealed an increased colocalization between C9orf72 and RAB7 and RAB11 compared with controls.[442] Recently, it was shown that the loss of C9orf72 might cause deregulation of autophagy via reduction of the activity of the negative regulator of autophagy MTOR, and the increase of the levels and nuclear translocation of TFEB, indicating that C9orf72 might serve as a negative regulator of autophagy, playing an important role in coupling the cellular metabolic state with autophagy regulation.[650]

An analysis of the role of autophagy in regulation of FUS-positive SGs was conducted in the same year by Ruy *et al.*[651] Here, it was shown that the FUS-positive SGs formed under oxidative stress in neurons bearing the ALS-linked $FUS^{R521C}$ mutation are colocalized to the zinc finger protein TRIM27 (tripartite motif containing 27) RFP-LC3-positive autophagosomes and are preferentially accumulated in autophagy-deficient neurons,[651] whereas rapamycin-enhanced autophagy is able to reduce the accumulation of FUS-positive SGs in an autophagy-dependent manner.[300,651] It was also indicated that autophagy activation not only reduces mutant FUS-positive SG accumulation, but also inhibits neurite fragmentation mediated by mutant FUS, and reduces cell death in response to oxidative stress in disease-associated cultured neurons.[300] Therefore, the enhancement of the autophagy activity leading to the reduction in the number of SGs containing mutant FUS can be neuroprotective,[651,652] whereas distortion of the autophagic process can contribute to the ALS-FTLD pathogenesis.[653]

## 6. Conjointness of the LLPT-based pathogenicity: Collective responsibility of proteins involved in PMLO formation

Because any PMLO (physiological or aberrant) represents a proteinaceous assemblage containing multiple proteins, LLPT-based pathologies represent a collective responsibility of proteins involved in PMLO formation. In essence, such kind of pathogenicity driven by aberrant PMLOs represents an example of a misdeed conducted by good guys assembled with bad company in the wrong place at the wrong time. Several examples below show how ALS-related proteins might cooperate, while affecting dynamics of PMLOs.

Although in the norm, TARDBP and FUS are preferentially localized in the nucleus, being transported there via the nuclear import receptors in a RAN (RAN, member RAS oncogene family) GTPase-dependent manner,[413] they also play a role in regulation of the RNA life cycle, being involved in the normal biogenesis of the intracytoplasmic RNA-containing PMLOs (e.g., SGs) in addition to regulation of the transcription, processing, transport, and stability of RNA.[392] In ALS and FTLD, both of these proteins mislocalize to the cytoplasm, where they form cytoplasmic inclusions, affect SG dynamics, and impair the RNA quality control system.[293] For example, the rate of TARDBP incorporation into the SGs is accelerated by the ALS-related mutations in this protein, and the mutated TARDBP forms larger SGs than the wild-type protein.[389] Similarly, FUS with the ALS-associated mutations efficiently localizes to SGs causing their assembly into larger structures.[505] However, TARDBP and FUS do not act alone, being engaged in interaction with each other, and this interaction is enhanced by the ALS-related mutations in TARDBP.[654] Furthermore, ATXN2 with intermediate-length polyglutamine expansions that serves as an ALS susceptibility factor[570-572] and is an SG constituent RNA-binding protein, is associated with both FUS-[580] and TARDBP-based pathologies.[577] For example, longer polyglutamine expansions in ATXN2 enhance interaction of this protein with TARDBP and increase the efficiency of the TARDBP cytoplasmic mislocalization.[570] These observations suggest that the ALS-FTLD pathology can be at least in part driven by the mislocalization of these 3 RNA-binding proteins to the cytoplasm that results in triggering the aberrant LLPTs leading to the formation of abnormal cytoplasmic PMLOs and promoting impairment of the RNA quality control system.[293,578]

TARDBP, FUS, and ATXN2 that interact with each other, potentially forming the core of the ALS-FTLD pathological cascade[578] are not exceptions to the rule, and other proteins associated with ALS-FTLD pathogenicity may also contribute to the aforementioned PMLO-related collective responsibility. For example, among the known C9orf72 targets are some proteins related to ALS, such as actin, HNRNPA1, HNRNPA2B1, and UBQLN2, and overexpression of this protein is accompanied by the formation of both nuclear C9orf72 aggregates and prominent cytoplasmic SGs containing HNRNPA1 and HNRNPA2B1.[442] It was also indicated that HNRNPA1 and HNRNPA2B1 can interact with TARDBP via their PrLDs.[364,367,552] Mutations of these proteins trigger their mislocalization from the nucleus to the cytoplasm, promote their aggregation and formation of sarcoplasmic inclusions, and cause multisystem proteinopathy and ALS.[552] Curiously, pathological mutations in HNRNPA1 and HNRNPA2B1 also stimulate mislocalization of wild-type TARDBP from the nucleus to the cytoplasm and result in formation of TARDBP-positive cytoplasmic inclusions. Typically, the inclusions formed by TARDBP, HNRNPA2B1, and HNRNPA1 are physically

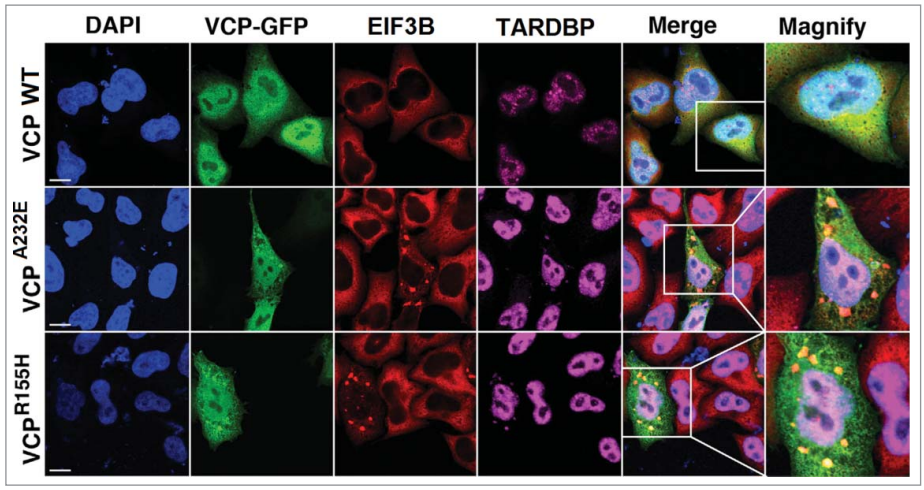


**Figure 8.** Effect of mutated forms of VCP (A232E and R155H) on the formation of constitutive SGs and the recruitment of TARDBP to those SGs. HeLa cells were transfected with plasmid expressing wild-type or mutant VCP-GFP forms and stained for the SG marker EIF3B and TARDBP. Overexpression of mutant but not wild-type VCP resulted in the formation of TARDBP-containing SGs. Scale bar: 10 $\mu$m. Reproduced with permission from ref. 655.

separated and only occasionally overlap.[551] Similarly, pathological mutations in VCP promote formation of cytoplasmic inclusions by the wild type versions of TARDBP, HNRNPA2B1, and HNRNPA1,[551] suggesting that the same phenotypic changes leading to TARDBP pathology and disease can be promoted by perturbations of the VCP, HNRNPA2B1, and HNRNPA1 pathways.[552] This important phenomenon is illustrated by Fig. 8 that represents the results of the analysis of the effect of mutant forms of VCP (A232E and R155H) on the formation of SGs and TARDBP recruitment to those SGs.[655] It is seen that the constitutive SGs containing TARDBP are formed only as a result of the overexpression of the mutated VCP, whereas no such effects were found when the wild-type VCP was overexpressed in HeLa cells.[655]

PURA can interact with the C-terminal region of the mutated FUS in an RNA-dependent manner, and these 2 proteins are colocalized in the SGs present in ALS patients.[505] Furthermore, accumulation of PURA within the intra-cytosolic SGs is enhanced in the presence of the $(GGGGCC)_{31}$ repeats[457] due to the ability of this protein to interact with the GGGGCC expansions of the *C9orf72* gene.[508] Interaction of FLCN with TARDBP can modulate shuttling of TARDBP between the nucleus and the cytoplasm,[521] thereby playing a role in regulation of SG dynamics. RBM45 shows some sequence similarity to TARDBP and FUS and forms both nuclear and cytoplasmic inclusions.[522] Furthermore, the homo-oligomeric form of this protein found in the cytoplasm can interact with both TARDBP and FUS, and accumulation of RBM45 in the cytoplasm leads to the formation of the TARDBP-positive SGs.[522] SS18L1 can both self-aggregate and interact with FUS.[533] Curiously, this protein serves as an illustration of the hypothesis that the ALS-FTLD pathology can be caused by misbehaving PMLOs other than SGs (e.g., compromised paraspeckle formation[544,545]). In fact, the dynamics, morphology, and integrity of paraspeckles can all be affected by aggregated SS18L1 due to the ability of this species to entrap other paraspeckle proteins.[533]

## 7. Concluding remarks: Linking PMLOs with neurodegeneration via protein intrinsic disorder

Observations presented in the previous sections clearly indicate that many proteins considered in this article and related to ALS and FTLD are either directly or indirectly involved in the LLPTs and formation of SGs and other PMLOs. Furthermore, being purified and analyzed in vitro, some of these proteins show high aggregation potential and the ability to undergo liquid-liquid demixing, leading to the formation of phase-separated droplets[656] or polymer hydrogels.[657] It is assumed that these membrane-less cellular compartments represent liquid droplets that phase separately from the cytoplasm and stably coexist with their environments.[658] Although typically LLPTs leading to the formation and disintegration of PMLOs represent normal physiological processes related to the organization of macromolecules into compartments needed for the proper functioning of a cell, abnormal liquid-liquid phase transitions and aberrant PMLOs can be associated with age-related neurodegenerative diseases,[658] via impairment of the cytoplasmic RNA quality control system[293] or some other related mechanisms.

It was also pointed out that LLPTs are often driven by IDPs containing low-complexity regions or domains.[205,206,226,269,271,657,659–666] In agreement with these earlier observations Fig. 3 shows that the majority of the proteins considered in this article that are related to ALS and FTLD contain long IDPRs. Although C9orf72 is the most ordered protein in this set, the pathological involvement of this protein to neurodegeneration is related to the hexanucleotide repeat expansions of the intronic region of the *C9orf72* gene leading to the bidirectional transcription of the expanded repeats associated with the generation of various sense and antisense repeat RNAs[433-436] that serve as mRNAs in RAN-translation to generate a series of DPRs.[434,436-439] All these DPRs including poly (GA), poly(GR), poly(GP), poly(PA), and poly(PR) proteins are highly disordered. Furthermore, many of the ALS- and FTLD-related proteins are able to interact with nucleic acids

and many protein partners, and many of them are involved in SG formation, and are associated with the assembly of SG-like inclusions in ALS and FTLD. For example, in addition to being involved in the formation of SGs, TARDBP and FUS are associated with several other PMLOs, such as DNA damage repair sites, paraspeckles, other RNA granules, and transport granules.[299,402,664,667,668]

In other words, intrinsic disorder might represent a feature unifying very different proteins in their relation to the pathogenesis of neurodegeneration via aberrant LLPTs and abnormal PMLOs. Therefore, it is likely that the "Dr. Jekyll–Mr. Hyde" behavior of proteins related to ALS and FTLD pathogenesis can at least in part be due to the ability of these proteins to be engaged in the intrinsic disorder-based LLPTs.

## Abbreviations

| | |
|---|---|
| APBB1/FE65 | amyloid beta precursor protein binding family B member 1 |
| ACTA2 | actin, alpha 2, smooth muscle, aorta |
| ACTB | actin beta |
| AD | Alzheimer disease |
| AKAP9/AKAP350 | A-kinase anchor protein 9 |
| ALS | amyotrophic lateral sclerosis |
| AMPA | $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| AMPK | AMP-activated protein kinase |
| ANG | angiogenin |
| APC | adenomatous polyposis coli |
| APOE | apolipoprotein E |
| ARF | ADP ribosylation factor |
| ARID1A/BAF250 | AT-rich interaction domain 1A |
| AS | alternative splicing |
| ATN1 | atrophin 1 |
| ATXN | ataxin |
| ATXN1 | ataxin 1 |
| ATXN2 | ataxin 2 |
| ATXN3 | ataxin 3 |
| ATXN7 | ataxin 7 |
| AXIN1 | axin 1 |
| BCL2 | BCL2, apoptosis regulator |
| BDNF | brain derived neurotrophic factor |
| BHD | Birt-Hogg-Dubé syndrome |
| BRCA1 | BRCA1, DNA repair associated |
| C9orf72 | chromosome 9 open reading frame 72 |
| CASC3/MLN51 | cancer susceptibility 3 |
| CCNA1 | cyclin A1 |
| CCNT1 | cyclin T1 |
| CCS | copper chaperone for superoxide dismutase |
| CDK | cyclin dependent kinase |
| CDKN1A/p21 | cyclin dependent kinase inhibitor 1A |
| CDKN1B/p27Kip1 | cyclin dependent kinase inhibitor 1B |
| CHCHD10 | coiled-coil-helix-coiled-coil-helix domain containing 10 |
| CHMP2B | charged multivesicular body protein 2B |
| CHRNB4 | cholinergic receptor nicotinic beta 4 subunit |
| CFTR | cystic fibrosis transmembrane conductance regulator |
| CHGA | chromogranin A |
| CHGB | chromogranin B |
| CNS | central nervous system |
| CPEB | cytoplasmic polyadenylation element binding protein |
| CREB | cAMP response element binding |
| CREBBP/CBP | CREB binding protein |
| CTA | cancer/testis antigen |
| CTF | C-terminal fragment |
| DDX6 | DEAD-box protein 6 |
| DDX19B/DBP5 | DEAD-box helicase 19B |
| DEAD box | protein with Walker B motif with the amino acid sequence D-E-A-D |
| Ded1 | ATP-dependent RNA helicase ded1 |
| DENND1B/connecdenn 2 | DENN domain containing 1B |
| DERL1 | derlin 1 |
| DMD | discrete molecular dynamics |
| DPR | dipeptide repeat proteins |
| E2F1 | E2 transcription factor 1 |
| EIF2 | eukaryotic translation initiation factor 2 |
| EIF2A | eukaryotic translation initiation factor 2A |
| EIF3 | eukaryotic translation initiation factor 3 |
| EIF4B | eukaryotic translation initiation factor 4B |
| EIF4G1/EIF4F | eukaryotic translation initiation factor 4 gamma 1 |
| EP300 | E1A binding protein p300 |
| ER | endoplasmic reticulum |
| ERN1/IRE1 | endoplasmic reticulum to nucleus signaling 1 |
| EWSR1 | EWS RNA binding protein 1 |
| fALS | familial form of ALS |
| F2 | coagulation factor II, thrombin |
| FAS | Fas cell surface death receptor |
| FASTK | Fas activated serine/threonine kinase |
| FET | protein family including FUS, EWSR1, and TAF15 |
| FLCN | folliculin |
| FMR1/FMRP | fragile X mental retardation 1 |
| FNIP | folluculin interacting protein |
| FTLD | frontotemporal lobar degeneration |
| FUS | FUS RNA binding protein |
| FXR1 | FMR1 autosomal homolog 1 |
| G3BP1 | G3BP stress granule assembly factor 1 |
| G3BP2 | G3BP stress granule assembly factor 2 |
| GLE1 | GLE1, RNA export mediator |

| | |
|---|---|
| GLFG | G-L-F-G repeat region of nucleoporin |
| GRN/PGRN | granulin precursor |
| GSK3 | glycogen synthase kinase 3 |
| HDAC | histone deacetylase |
| HNE | 4-hydroxynonenal |
| HOA | homo-oligomer assembly domain |
| HNRNP | heterogeneous nuclear ribonucleoprotein |
| HNRNPA1 | heterogeneous nuclear ribonucleoprotein A1 |
| HNRNPA2B1 | heterogeneous nuclear ribonucleoprotein A2/B1 |
| HPV | human papillomavirus |
| HTT | huntingtin |
| IAPP/amylin | islet amyloid polypeptide |
| IDP | intrinsically disordered protein |
| IDPR | intrinsically disordered protein region |
| KEAP1 | kelch like ECH associated protein 1 |
| LCCS1 | lethal congenital contracture syndrome 1 |
| LAAHD | lethal arthrogryposis with anterior horn cell disease |
| LIMK | LIM domain kinase |
| LLPT | liquid-liquid phase transition |
| MAP1LC3B | microtubule associated protein 1 light chain 3 beta |
| MAP2K7/MKK7 | mitogen-activated protein kinase kinase 7 |
| MAPK3/ERK1 | mitogen-activated protein kinase 3 |
| MAPK8/JNK1 | mitogen-activated protein kinase 8 |
| MAPT | microtubule associated protein tau |
| MBP | myelin basic protein |
| MFD | multifunctional domain |
| MoRF | molecular recognition feature |
| mtSOD1 | mutant SOD1 |
| MTORC1 | mechanistic target of rapamycin complex 1 |
| NEFH | neurofilament heavy |
| NEFL/NFL | neurofilament light |
| NEK1 | NimA related kinase 1 |
| NES | nuclear export signal |
| NimA | never in mitosis A |
| NLS | nuclear localization signal |
| NPC | nuclear pore complex |
| NPM1 | nucleophosmin 1 |
| NFE2L2 | nuclear factor, erythroid 2 like 2 |
| NTD | N-terminal domain |
| NUP155 | nucleoporin 155 |
| NUPL2 | nucleoporin like 2 |
| OPTN | optineurin |
| OS | oxidative stress |
| PABPC1/PABP1 | poly(A) binding protein cytoplasmic 1 |
| P-body | processing body |
| PBP | progressive bulbar palsy |
| PD | Parkinson disease |
| PFN1 | profilin 1 |
| *pfy1Δ* | yeast cells lacking profilin |
| PLS | primary lateral sclerosis |
| PMA | progressive muscular atrophy |
| PMLO | proteinaceous membrane-less organelle |
| PPI | protein-protein interaction |
| PrLD | prion-like domain |
| PRNP/PrP | prion protein |
| PRMT1 | protein arginine methyltransferase 1 |
| PTEN | phosphatase and tensin homolog |
| PTM | posttranslational modification |
| PURA | purine rich element binding protein A |
| PV | poliovirus |
| RAB | RAB, member RAS oncogene family |
| RACK1 | receptor for activated C kinase 1 |
| RAD23B/HR23B | RAD23 homolog B, nucleotide excision repair protein |
| RAN | RAN, member RAS oncogene family |
| RAN-translation | repeat-associated non-ATG translation |
| RB1 | RB transcriptional corepressor 1 |
| RBM45 | RNA binding motif protein 45 |
| RBP | RNA binding protein |
| RHOA | ras homolog family member A |
| RNP | ribonucleoprotein |
| Rnq1 | [PIN+] prion protein RNQ1 |
| ROCK1 | Rho associated coiled-coil containing protein kinase 1 |
| ROS | reactive oxygen species |
| RPTOR/Raptor | regulatory associated protein of MTOR complex 1 |
| RRM | RNA-recognition motif |
| RPS6KA3/RSK2 | ribosomal protein S6 kinase A3 |
| sALS | sporadic form of ALS |
| SCA2 | spinocerebellar ataxia 2 |
| SG | stress granule |
| SIRT | sirtuin |
| SLC1A2/EAAT2 | solute carrier family 1 member 2 |
| SMARCA4/BRG1 | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 |
| SMCR8 | Smith-Magenis syndrome chromosome region, candidate 8 |
| SMN | survival motor neuron protein |
| SOD1 | superoxide dismutase 1 |
| SP1 | Sp1 transcription factor |
| SPN/CD43 | sialophorin |
| SQSTM1/p62 | sequestosome 1 |
| SS18L1/CREST | SS18L1, nBAF chromatin remodeling complex subunit |
| Sup35 | eukaryotic peptide chain release factor GTP-binding subunit |
| TAF15 | TATA-box binding protein associated factor 15 |
| TANK | TRAF family member associated NFKB activator |

| | |
|---|---|
| TARDBP/TDP-43 | TAR DNA binding protein |
| TBK1 | TANK binding kinase 1 |
| TBP | TATA-box binding protein |
| TFEB | transcription factor EB |
| TIA1 | TIA1 cytotoxic granule associated RNA binding protein |
| TIAL1/TIAR | TIA1 cytotoxic granule associated RNA binding protein like 1 |
| TP53 | tumor protein p53 |
| TP53BP/ASPP | tumor protein p53 binding protein |
| TRAF2 | TNF receptor associated factor 2 |
| TRIM27 | tripartite motif containing 27 |
| UBC | ubiquitin C |
| UBQLN2 | ubiquilin 2 |
| UNC119/RG4 | unc-119 lipid binding chaperone |
| UPS | ubiquitin-proteasome system |
| VCP | valosin containing protein |
| VDAC1 | voltage dependent anion channel 1 |
| VEGF | vascular endothelial growth factor |
| WDR41 | WD repeat domain 41 |
| WDR62 | WD repeat domain 62 |
| WT-SOD1 | wild-type SOD1 |
| YBX1/YB1 | Y-box binding protein 1 |
| YWHAB | tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta |
| ZFP36/TTP | ZFP36 ring finger protein |

## Conflicts of interest

The author declares no conflict of interest.

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