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TDP-43 Aggregation In Neurodegeneration: Are Stress Granules The Key?

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Abstract

The RNA-binding protein TDP-43 is strongly linked to neurodegeneration. Not only are mutations in the gene encoding TDP-43 associated with ALS and FTLT, but this protein is also a major constituent of pathological intracellular inclusions in these diseases. Recent studies have significantly expanded our understanding of TDP-43 physiology. TDP-43 is now known to play important roles in neuronal RNA metabolism. It binds to and regulates the splicing and stability of numerous RNAs encoding proteins involved in neuronal development, synaptic function and neurodegeneration. Thus, a loss of these essential functions is an attractive hypothesis regarding the role of TDP-43 in neurodegeneration. Moreover, TDP-43 is an aggregation-prone protein and, given the role of toxic protein aggregates in neurodegeneration, a toxic gain-of-function mechanism is another rational hypothesis. Importantly, ALS related mutations modulate the propensity of TDP-43 to aggregate in cell culture. Several recent studies have documented that cytoplasmic TDP-43 aggregates co-localize with stress granule markers. Stress granules are cytoplasmic inclusions that repress translation of a subset of RNAs in times of cellular stress, and several proteins implicated in neurodegeneration (i.e. Ataxin-2 and SMN) interact with stress granules. Thus, understanding the interplay between TDP-43 aggregation, stress granules and the effect of ALS-associated TDP-43 mutations may be the key to understanding the role of TDP-43 in neurodegeneration. We propose two models of TDP-43 aggregate formation. The “independent model” stipulates that TDP-43 aggregation is independent of stress granule formation, in contrast to the “precursor model” which presents the idea that stress granule formation contributes to a TDP-43 aggregate “seed” and that chronic stress leads to concentration-dependent TDP-43 aggregation.

Keywords

TARDBP; TDP-43; FUS; stress granules; FTD; FTLT; frontotemporal lobar degeneration; ALS; amyotrophic lateral sclerosis; neurodegeneration; independent model; precursor model

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1. Introduction

1.1 TDP-43: an RNA-binding protein pathologically and genetically linked to amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD)

TAR DNA binding protein 43 (TDP-43) is a histopathological marker of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD)(Arai et al., 2006; Neumann et al., 2006), as well as multiple other neurodegenerative diseases (Alzheimer's(Amador-Ortiz et al., 2007), Parkinson's(Lin and Dickson, 2008), and Huntington's(Schwab et al., 2008) diseases, hippocampal sclerosis(Amador-Ortiz et al., 2007), and dementia with Lewy's bodies(Lin and Dickson, 2008); reviewed in (Lagier-Tourenne et al., 2010)) . In ALS and FTLD post-mortem tissue, TDP-43 inclusion morphology ranges from skein-like to dense bodies. This pathology is present in neurons and glia, both in gray and white matter. TDP-43 protein in these inclusions is pathologically altered, i.e. mislocalized, aggregated, ubiquitinated and truncated. Also, TDP-43 is genetically linked(Benajiba et al., 2009; Borroni et al., 2009; Kabashi et al., 2008b; Rutherford et al., 2008; Sreedharan et al., 2008) to both ALS and FTLD, indicating a role in pathogenesis.

TDP-43 is encoded by the TARDBP gene on chromosome 1p36 into a 414 amino acid, 43 kDa protein. Its name was originally derived from the fact that it bound the transactivation response region (TAR) of HIV DNA(Ou et al., 1995). It was afterwards found to bind pre-mRNA at UG-rich sequences(Buratti and Baralle, 2001; Buratti et al., 2004). It is highly conserved, ubiquitously expressed, and essential for embryonic development(Sephton et al., 2010). Structurally, TDP-43 belongs to a family of RNA-binding proteins known as heterogeneous nuclear ribonucleoproteins (hnRNPs)(Buratti and Baralle, 2001). Many hnRNPs bind their mRNA targets to repress the inclusion of exons, thereby modulating splicing patterns (reviewed in (Dreyfuss et al., 2002)). Likewise, TDP-43 influences alternative splicing, including isoforms of genes that regulate neuronal development or that are implicated in neurodegeneration(Polymenidou et al., 2011; Tollervey et al., 2011). TDP-43 also binds to its own mRNA(Sephton et al., 2011) and regulates its own levels by a feedback loop(Ayala et al., 2011b; Sephton et al., 2010).

TDP-43 has five functional regions, including two RNA recognition motifs (RRM1 and RRM2), one glycine-rich region (GRR), as well as a nuclear localization signal (NLS) and nuclear export signal (NES) that mediate nucleocytoplasmic shuttling(Ayala et al., 2008) (Fig. 1). Both RRM1 and RRM2 enable TDP-43 to interact with single-stranded RNA and DNA(Buratti and Baralle, 2001). In contrast, the GRR mediates protein:protein interactions with other hnRNPs(Buratti et al., 2005). Within the GRR lies a Q/N-rich region that is aggregation-prone and is hypothesized to confer prion-like properties(Fuentealba et al., 2010; Polymenidou and Cleveland, 2011). Importantly, the GRR spans the region where the majority of genetic mutations have been identified(Del Bo et al., 2009; Kabashi et al., 2008a; Kuhnlein et al., 2008; Rutherford et al., 2008; Van Deerlin et al., 2008). Currently, over 40 TARDBP mutations have been found in ALS patients, while 3 have been found in FTLD patients(Borroni et al., 2009; Gitcho et al., 2009; Kovacs et al., 2009).

1.2 TDP-43 in neurodegeneration

How TDP-43 contributes to the neurodegenerative process is unclear, but a few hypotheses have been proposed: (1) a toxic cytoplasmic gain of function, or (2) a nuclear loss of function. The nuclear loss of function hypothesis posits that TDP-43 has distinct nuclear roles that are lost following sequestration in cytoplasmic inclusions. The loss of function hypothesis is attractive because TDP-43 is now recognized to target thousands of RNAs, including neuronal-specific RNAs implicated in neurodegenerative disorders(Polymenidou et al., 2011; Sephton et al., 2011; Strong et al., 2007; Tollervey et al., 2011; Wang et al.,

2008). However, the toxic cytoplasmic gain of function hypothesis is also enticing because TDP-43 overexpression and aggregation is toxic to a wide variety of cells spanning yeast(Johnson et al., 2008) to mammalian cells(Winton et al., 2008). Importantly, ALS-related mutations alter cytoplasmic aggregation propensity of TDP-43(Dewey et al., 2011), and TDP-43⁺ inclusions are the pathological hallmark of FTLTDP and ALS.

1.3 Pathological TDP-43 aggregates

The origin of TDP-43⁺ histopathological inclusions *in vivo* is poorly understood. In cell culture, TDP-43 localizes to cytoplasmic RNA granules including, dendritic processing bodies(Wang et al., 2008) and stress granules(Colombrita et al., 2009; Dewey et al., 2011; Liu-Yesucevitz et al., 2010; McDonald et al., 2011). These RNA granules may be precursors to the pathologically altered cytoplasmic aggregates. In a recent study, ubiquitinated TDP-43⁺ aggregates in ALS and FTLTDP patient spinal cord and brain, respectively, were found to colocalize with T-cell intracellular antigen-1 (TIA-1) and eukaryotic initiation factor 3 (eIF3), known stress granule markers(Liu-Yesucevitz et al., 2010). Yet, these findings contradict earlier reports(Colombrita et al., 2009; Neumann et al., 2007). While TDP-43 is evidently a stress responsive protein, it not clear whether pathological TDP-43 inclusions arise from stress granules. In this review, we address the function of stress granules, how wild-type and mutant TDP-43 localizes to these structures, affects their formation and disassembly and the possible pathological significance of these findings.

2. Stress granule biology

2.1 Composition and assembly of stress granules

Stress granules are transient cytoplasmic structures that are formed in response to cellular stress and act as sorting stations for mRNAs(Nover et al., 1989), reviewed in (Anderson and Kedersha, 2008; Buchan and Parker, 2009). They are part of a spectrum of cytoplasmic ribonucleoprotein particles that also includes processing(P-) bodies(Bashkirov et al., 1997; Eystathiou et al., 2002), neuronal (transport) RNPs(Barbee et al., 2006), and dendritic P-bodies(Cougot et al., 2008). Stress granules contain mRNAs, the 40S subunit of ribosomes and various (>30) proteins (see below). The composition of P-bodies, where mRNA decapping and degradation likely takes place(Sheth and Parker, 2003), is similar and mRNAs and ribonucleoproteins are thought to shuttle between them and stress granules(Brengues et al., 2005; Buchan and Parker, 2009). However, some proteins localize exclusively to P-bodies (i.e., decapping proteins 1a and 2, [DCP1a and DCP2])(Kedersha et al., 2005), or specifically to stress granules (see below). P-bodies are often observed juxtaposed to stress granules, but also are present in cells not under stress. Neuronal P-body-like structures (transport RNPs and dendritic P-bodies) are transported by motor proteins to dendrites and their composition is activity-dependant; these structures are most likely involved in local translation at the dendrites(Barbee et al., 2006). TDP-43 can localize to both dendritic P-bodies(Wang et al., 2008) and stress granules.

Cytoplasmic stress granules form when translation is stalled at the initiation step. The 48S preinitiation complex is normally bound by the ternary complex (eIF2 α -GTP-tRNA^{Met}) to initiate translation. During stress, eukaryotic initiation factor 2 alpha (eIF2 α) is phosphorylated, and this prevents assembly of the ternary complex(Kaufman et al., 1989). This can occur when a cell is exposed to specific chemicals (puromycin(Kedersha et al., 2000)) or environmental stressors (oxidative stress(Kedersha et al., 2002), heat shock(Nover et al., 1989), hyperosmolarity(Dewey et al., 2011), or viral infection(Eslatine et al., 2004; Mazroui et al., 2006)). An eIF2 α phosphorylation-independent pathway, which is activated in response to heat shock(Farny et al., 2009; Grousl et al., 2009; Kramer et al., 2008) or

inhibition of translation initiation factors eIF4A and eIF46(Mazroui et al., 2006; Mokas et al., 2009) also results in stress granule formation.

Stress granule composition and morphology varies in a cell and stress dependent manner, however a number of components are consistent across all types of stress(Buchan et al., 2011; Guil et al., 2006). Conserved or “core” stress granule components include TIA-1(Gilks et al., 2004; Kedersha et al., 2000), TIA-1 related protein (TIAR)(Kedersha et al., 1999), and stalled translation initiation complex factors 3 and 4G (eIF3 and eIF4G, respectively)(Kedersha et al., 2002; Kimball et al., 2003). TIA-1 and TIAR are two nucleocytoplasmic shuttling proteins that are localized to the nucleus in unstressed cells. Upon stress, TIA-1 and TIAR shuttle to the cytoplasm where they aggregate(Kedersha et al., 1999). Once the stress granule is formed by obligatory components, additional proteins are recruited to these structures. Examples of non-core stress granule components include RNA-binding proteins (hnRNP A1, FUS and TDP-43)(Bosco et al., 2010; Guil et al., 2006), helicases (p54/Rck/DDX6)(Wilczynska et al., 2005), and exonucleases (XRN1)(Kedersha et al., 2005). An up-to-date list is given in (Buchan and Parker, 2009).

2.2 Function of stress granules

Stress granules are dynamic structures that are thought to triage (sort) mRNAs during stress (Fig. 2) (Anderson and Kedersha, 2008; Kedersha and Anderson, 2002; Nover et al., 1989). The triage process determines mRNA fate: translation, sequestration in the stress granule or degradation. mRNAs triaged for translation or degradation do not remain in the stress granule: translation takes place in polysomes, while degradation occurs in P-bodies. mRNA recruitment to stress granules is not random(Piecyk et al., 2000). For example, nutrient deprivation leads to association of core stress granule components TIA-1/TIAR with mRNAs containing 5'-terminal oligopyrimidine tracts(Damgaard and Lykke-Andersen, 2011), including mRNAs encoding PABPC1, Rpl23a, and rpl36. mRNAs encoding calmodulin 2 and β -actin also associate with TIA-1/TIAR. (Interestingly, PABPC1, calmodulin 2 and β -actin mRNAs are also bound by TDP-43 in neurons(Sephton et al., 2011).) The overall effect of this mRNA sequestration is probably to slow down growth, translation and ATP consumption. This, in turn, may help the cell survive a period of stress.

2.2 Disassembly of stress granules

Translation is subdivided into three steps: initiation, elongation, and termination. Stress granule assembly occurs when translation initiation is inhibited(Kedersha et al., 2002). However, inhibition at the elongation step both prevents stress granule assembly and disassembles already-present stress granules. Stress granule disassembly naturally takes place following stress removal, a process that occurs as quickly as 15 minutes in some cells. Alternatively, chemicals inhibiting the elongation step (cycloheximide and emetine) (Kedersha and Anderson, 2009), and overexpression of certain proteins (i.e., stauferin) can result in stress granule disassembly(Thomas et al., 2009).

3. TDP-43 in stress granule biology

3.1 TDP-43 is not an obligatory stress granule component

Cell culture models have been used to discern two determinants of TDP-43 localization to stress granules: the stressor and the cell type. Stressors that direct TDP-43 to stress granules in cell culture include: heat shock, oxidative stress, osmotic stress, serum deprivation, ubiquitin-proteasome inhibition, thapsigargin (endoplasmic reticulum stress) and paraquat (a herbicide)(Colombrita et al., 2009; Dewey et al., 2011; Freibaum et al., 2010; Liu-Yesucevitz et al., 2010; McDonald et al., 2011; Meyerowitz et al., 2011)(Table 1). TDP-43 also localizes to stress granules in mixed primary glial cultures following sorbitol (osmotic

and oxidative) stress(Dewey et al., 2011), and *in vivo* in axotomized mouse motor neurons(Moisse et al., 2009).

On the other hand, TDP-43 failed to localize to stress granules in neural cell lines (Neuro2a and SH-SY5Y) treated with epoxomicin (proteasome inhibitor) and thapsigargin(Ayala et al., 2011a). In addition, arsenite stress in HeLa cells directs TDP-43 to stress granules, but not in Hek293T cells(Dewey et al., 2011). These findings indicate that TDP-43 is not an obligatory stress granule component, meaning TDP-43 is responsive to specific stressors, but not to all. A summary of TDP-43's response to stress is presented in table 1.

3.2 TDP-43 protein levels modulate stress granule formation, size and disassembly

There are multiple routes to stress granule assembly, environmental stress being the best characterized route. However, unstressed cells can also form stress granules when an “obligatory component” is overexpressed. The obligatory components TIA-1 and TIAR aggregate in a concentration-dependent manner mediated by their prion-like domains(Gilks et al., 2004). The aggregation of these proteins is said to “nucleate” stress granule assembly. In contrast, overexpression of wild-type TDP-43 fails to nucleate stress granules(Colombrita et al., 2009; Dewey et al., 2011; Liu-Yesucevitz et al., 2010). However, overexpression of TDP-43 with a defective NLS or NES results in cytoplasmic or nuclear aggregates, respectively(Winton et al., 2008). These cytoplasmic aggregates were recently shown to co-stain with stress granule markers(Liu-Yesucevitz et al., 2010).

TDP-43 protein levels modulate stress granule assembly. TDP-43 knockdown has been shown to delay (not prevent) stress granule assembly(McDonald et al., 2011). The proposed mechanism is that TDP-43 knockdown reduces TIA-1 protein levels, a protein that nucleates stress granules. However, TDP-43 knockdown did not alter the levels of another stress granule nucleator Ras GAP-associated endoribonuclease (G3BP)(McDonald et al., 2011), which may explain why stress granule formation was not completely abolished. Another study investigated the effect of TDP-43 overexpression on stress granule formation. As pathological mutants are now understood to be more stable than wild-type(Ling et al., 2010), increased expression levels are another plausible mechanism mediating TDP-43 pathology. Cells overexpressing wild-type protein were contrasted with familial mutants causing ALS: the stress response was distinct in that familial mutants localized to stress granules with a faster timecourse, and assembled into larger stress granules than the wild-type stress response(Dewey et al., 2011). This study, and others, have also confirmed that overexpression of both familial and sporadic mutants results in more stress granules formed per cell(Liu-Yesucevitz et al., 2010). Stress granule assembly therefore, is affected by both TDP-43 knockdown and overexpression.

3.3 TDP-43 structure and JNK signaling determine localization to stress granules

Localization of TDP-43 to stress granules requires the RRM1 domain and a segment of the GRR(Colombrita et al., 2009; Dewey et al., 2011; Freibaum et al., 2010). Specifically, one report found residues 216–315 to be necessary for this association, while another report narrowed these residues to 267–324 (Fig. 1)(Colombrita et al., 2009; Dewey et al., 2011). Also, c-Jun N-terminal kinase (JNK) pathway activation is necessary for stress granule association in an arsenite (oxidative) stress model(Meyerowitz et al., 2011). This finding is interesting, as it raises many questions about the relationship between TDP-43 and JNK signaling, such as whether TDP-43 is a direct or indirect JNK target.

4. Stress granule biology and neurodegeneration

4.1 Stress granule-associated proteins linked to neurodegenerative diseases

RNA processing errors and dysfunctional stress responses may be key mediators of both ALS and FTLT pathogenesis. Early evidence for this hypothesis came from the ALS field where increased oxidative stress was shown to recapitulate key aspects of this disease (Abe et al., 1995; Shaw et al., 1995). Currently, support for this hypothesis is mounting as more RNA-binding proteins are genetically-linked to ALS and other neurodegenerative diseases. Examples of this include ataxin-2 (which is mutated in spinocerebellar ataxia) (Imbert et al., 1996; Pulst et al., 1996; Sanpei et al., 1996), survival motor neuron (SMN, spinal muscular atrophy) (Thompson et al., 1995), fragile X mental retardation protein (FMRP, fragile-X syndrome) (Verkerk et al., 1991), in addition to fused in sarcoma (FUS, ALS and FTLT) (Kwiatkowski et al., 2009; Vance et al., 2009), angiogenin (ANG, ALS and PD) (Greenway et al., 2006; van Es et al., 2011) and TDP-43 (ALS and FTLT) (Rutherford et al., 2008; Sreedharan et al., 2008). The relationship between stress granule size, activation of cell death pathways, and the generation of pathological aggregates remains unknown. More is understood about the relationship between the stress-activated ribonuclease angiogenin, stress granules and the pathophysiology of ALS. Briefly, angiogenin cleaves transfer RNA (tRNA) to generate stress-induced fragments called tiRNAs (5' and 3'). Specifically, the 5' tiRNAs inhibit translation initiation using a novel stress pathway. These 5' tiRNAs interact with stress granule proteins (TDP-43, eIF4G and eIF4E) and are capped by a 5' monophosphate. This capping process is necessary for optimal stress granule assembly (Emara et al., 2010; Ivanov et al., 2011; Yamasaki et al., 2009). Analysis of angiogenin-associated ALS mutants has revealed a complete loss of function resulting from deficient ribonuclease activity (Wu et al., 2007). Thus, ALS pathophysiology may result from the inability of mutant angiogenin to initiate this novel stress pathway. As TDP-43 is a known interactor of 5' tiRNAs, future studies addressing if and how pathological TDP-43 modulates this stress pathway would significantly advance our understanding of RNA processing errors in the pathophysiology of ALS.

4.2 Stress granules and signaling pathways

Stress granules have been suggested to suppress apoptosis by suppressing the stress-activated mitogen activated protein kinase (MAPK) pathway (Arimoto et al., 2008), blocking pro-inflammatory signaling between TNF- α and NF- κ B through sequestration of TRAF2 (Kim et al., 2005), or by sequestration of rho-associated coiled coil containing protein kinase 1 (ROCK1) to prevent its interaction with pro-apoptotic c-Jun N-terminal kinase (JNK) pathway members (Tsai and Wei, 2010). This protective response is also beginning to be documented in the pyramidal neurons of Alzheimer's patients, where an inverse correlation exists between neurons forming stress granules and neurons forming neurofibrillary tangles (Castellani et al., 2011).

4.3 Modulators of stress granule formation and disassembly

Non-core stress granule components may affect stress granule formation and disassembly. Among stress granule associated proteins that are also involved in neurodegeneration, Ataxin-2 levels interfere with stress granule assembly (Nonhoff et al., 2007), as does FMRP (Didiot et al., 2009). Importantly, FUS, the other RNA-binding protein linked to ALS and FTLT, also modulates stress granule assembly when the pathological mutant is overexpressed (Bosco et al., 2010; Dormann et al., 2010).

The formation and disassembly of stress granules is in part determined by microtubule stability (Chernov et al., 2009), the motor proteins dynein and kinesin (Loschi et al., 2009; Tsai et al., 2009), RhoA/ROCK1 (Tsai and Wei, 2010) signaling and Grb7/FAK

signaling(Tsai et al., 2008). Because TDP-43 pathological mutants respond to stress by localizing to larger stress granules in cell culture, it is plausible that one of these processes is altered. It is particularly interesting that microtubule stability modulates stress granule size, as it is also implicated in neurodegeneration through microtubule-associated protein tau (MAPT or tau protein)(Dumanchin et al., 1998).

5. Summary and Perspective

TDP-43 is a stress-responsive RNA-binding protein linked to ALS and FTLT. Testable hypotheses were initially limited by our understanding of the basic cellular biology of TDP-43. However, the recent identification of thousands of novel TDP-43 RNA targets has greatly expanded our understanding of TDP-43 function and led to more refined hypotheses. Because FUS (another RNA binding protein) is also pathologically altered in and genetically linked to both ALS and FTLT, one of the strongest hypotheses is that altered RNA metabolism underlies pathogenesis. On the other hand, the cytoplasmic gain of function hypothesis posits that TDP-43 inclusions are toxic and mediate cell death. The recent finding that TDP-43 inclusions co-localize with some of the stress granules markers suggests that an altered stress response is a plausible explanation for the ALS-related phenotype i.e. cytoplasm accumulation, aggregation and reduced solubility.

Given what is known about TDP-43 biology, we propose two models for TDP-43 aggregation. The first model is that TDP-43 aggregation is independent of stress granule formation. In this “independent model”, stress granules form in response to stress and once the stress is removed; stress granules dissociate (Fig. 3). Alternatively, TDP-43 aggregates form due to an unknown factor or form as the result of a dying cell (Fig. 3). In the second model, we propose that stress granules are precursors to TDP-43 aggregation (Fig. 3). This “precursor model”, which we favor, stipulates that stress granule formation contributes to a TDP-43 aggregate “seed” and chronic stress (i.e. genetic mutations, environmental factors) may lead to concentration-dependent TDP-43 aggregation. TDP-43 with ALS-associated mutations forms larger stress granules which may indicate that concentration-dependent TDP-43 aggregation is achieved more readily (Fig. 3). The “precursor model” does not address the exact mechanism by which additional stress could lead to TDP-43 aggregates, however, the colocalization of TDP-43 with TIA-1 and eIF3 in ALS and FTLT patients suggests that stress granules may be involved to some degree (Liu-Yesucevitz et al., 2010). These models do not consider the toxicity proposed by the prion model of TDP-43 pathogenesis, which is the hypothesis that TDP-43 aggregates can “infect” adjacent cells and cause cellular aggregates and toxicity(Polymeridou and Cleveland, 2011). However, our “precursor model” is in line with the prion model in that TDP-43 aggregation occurs in a concentration-dependent manner.

Some TDP-43 mutations, upon stress, exhibit a distinct stress response relative to the wild-type protein. This response is marked by a more rapid formation and larger size of stress granules (depicted in Fig. 3). It should be noted that this observation was made in cells overexpressing these mutant proteins. Is this pathophysiologically significant, or just an overexpression artifact? Is this apparent change in SG size a reflection of the recently proposed prion-like properties of TDP-43 and aggregation-proneness of ALS-associated mutants? Does stress granule size alter cellular signaling pathways, perhaps by sequestering key components? Conceivably, the answers to these questions will resolve the paramount question of whether stress granules are the key to TDP-43 aggregation and neurodegeneration.

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Highlights

- We review the relationship of stress granules and TDP-43 pathological aggregation.
- We propose two possible models for TDP-43 aggregate formation.
- In one model, TDP-43 aggregation is “independent” of stress granule formation.
- In another model, stress granules are “precursors” to TDP-43 aggregation.

Figure 1. TDP-43 structural regions
TDP-43 has five functional regions including two RNA recognition motifs (RRM1 and RRM2), a nuclear localization signal (NLS) and nuclear export signal (NES) that mediates nucleocytoplasmic shuttling, and a glycine rich region (GRR) that mediates protein:protein interactions. Below the schematic, the prion-like Q/N rich region has been highlighted, in addition to the location of the majority of ALS-associated mutations. A structural region mediating TDP-43 association with stress granules (SGs), as identified in two independent studies, also spans part of the prion-like Q/N rich sequence.

Figure 2. Stress granule assembly and disassembly

Phase I. In response to stress, translation in the cytoplasm stops. The large ribosomes run off polysomes, while RNA-binding proteins and small ribosome subunits remain bound to stalled transcripts. *Phase II.* Stress granule (SG) nucleation is mediated by core stress granule proteins (i.e. TIA1, G3P, eIF3 and PABP) (purple) followed by recruitment of TDP-43 (orange), FUS (green), and proteins (blue) into the stress granule. Composition of the stress granule changes as the mRNA is triaged or sorted to different fates. *Phase III.* After stress is removed, stress granules disassemble. As the translation of sequestered transcripts resume, RNA-binding proteins dissociate.

Figure 3. Proposed models of TDP-43 aggregate formation

In Model 1, we propose that formation of TDP-43 aggregation is independent of stress granule formation. In this “independent model”, stress granules (SG) form in response to stress (left) and once the stress is removed; stress granules dissociate (stress granule core proteins, purple; TDP-43, orange; FUS, green; other proteins, blue). Alternatively, TDP-43 aggregates form due to an unknown factor or form as the result of a dying cell (right). In Model 2, we propose the stress granule is the precursor to aggregation. This “precursor model,” stipulates that stress granule formation contributes to a TDP-43 aggregate “seed” and chronic stress (i.e. genetic mutations, environmental factors) may lead to concentration-dependent TDP-43 aggregation. TDP-43 mutations (x) form larger stress granules, which may mean concentration-dependent TDP-43 aggregation is achieved more readily.

Table 1
TDP-43 localization to stress granules is dependent on cell type and stressor

Recent studies confirm TDP-43 localizes to stress granules (cell culture and *in vivo*). However, TDP-43 is not an obligatory stress granule component, as TDP-43 localization to stress granules depends on cell type and stressor.

System:	Stressor/dose/duration:	TDP loc to SG?	Reference:
Immortalized cells			
BE-M17	serum deprivation, 1 hr	Y	Liu-Yesucevitz
	sodium arsenite, 0.5 mM, 1 hr	Y	Liu-Yesucevitz
Hek293T	sorbitol, 400 mM, 0–4 hrs	Y	Dewey
	sodium arsenite, 0.5 mM, 1 hr	N	Dewey
HeLa	MG-132, 50 μ M, 3 hrs	Y	Freibaum
	sodium arsenite, 0.5 mM, 30 min	Y	McDonald
	thapsigargin, 1 μ M, 50 min	Y	McDonald
	heat shock, 43 C, 30 min	Y	McDonald
	paraquat, 1 mM, overnight	Y	Meyerowitz
Neuro2a	hydrogen peroxide, 10 μ M, 0–4 hrs	N	Ayala
	thapsigargin, 5 μ M, 0–4 hrs	N	Ayala
	epoxomicin, 2.5 μ M, 0–4 hrs	N	Ayala
NSC34	sodium arsenite, 0.5 mM, 0.5 hr	Y	Colombrita
	heat shock, 44 C, 0.5 hr	Y	Colombrita
SH-SY5Y	hydrogen peroxide, 10 μ M, 0–4 hrs	N	Ayala
	thapsigargin, 5 μ M, 0–4 hrs	N	Ayala
	epoxomicin, 2.5 μ M, 0–4 hrs	N	Ayala
	arginine, 1mM, overnight	N	Meyerowitz
	SIN-1, 0.1mM, overnight	N	Meyerowitz
	paraquat, 1mM, overnight	Y	Meyerowitz
U87MG	paraquat, 1 mM, overnight	Y	Meyerowitz
Primary cells:			
mixed glial culture	sorbitol, 400 mM, 1 hr	Y	Dewey
spinal cord organotypic slices	THA, 100 μ M, 21 days	N	Ayala
Animal models:			
motor neurons	sciatic axotomy, post-injury day 7	Y	Moisse