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Maria Udan

*Washington University School of Medicine in St. Louis*

Robert H. Baloh

*Washington University School of Medicine in St. Louis*

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## Implications of the prion-related Q/N domains in TDP-43 and FUS

Maria Udan<sup>1</sup> and Robert H. Baloh<sup>1,2,\*</sup>

<sup>1</sup>Department of Neurology; Neuromuscular Division; <sup>2</sup>Hope Center for Neurological Disorders; Washington University; St. Louis, MO USA

**A**myotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are clinically overlapping neurodegenerative disorders whose pathophysiology remains incompletely understood. ALS initiates in a discrete location and typically progresses in a pattern consistent with spread of the degenerative process to involve neighboring regions of the motor system, although the basis of the apparent “spread” remains elusive. Recently mutations in two RNA binding proteins, TDP-43 and FUS, were identified in patients with familial ALS. In addition to being involved in numerous events related to RNA metabolism, each forms aggregates in neurons in ALS and FTLD. Recent evidence also indicates that both TDP-43 and FUS contain prion-related domains rich in glutamine (Q) and asparagine (N) residues, and in the case of TDP-43 this is the location of most disease causing mutations. This review discusses the potential relevance of the prion-related domains in TDP-43 and FUS in normal physiology, pathologic aggregation and disease progression in ALS and FTLD.

**Key words:** amyotrophic lateral sclerosis, frontotemporal dementia, motor neuron disease, protein aggregation, RNA metabolism, prion domain

**Abbreviations:** ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; TDP-43, TAR DNA binding protein 43 kD; FUS, fused in sarcoma; RBD-Gly, RNA binding domain, glycine rich

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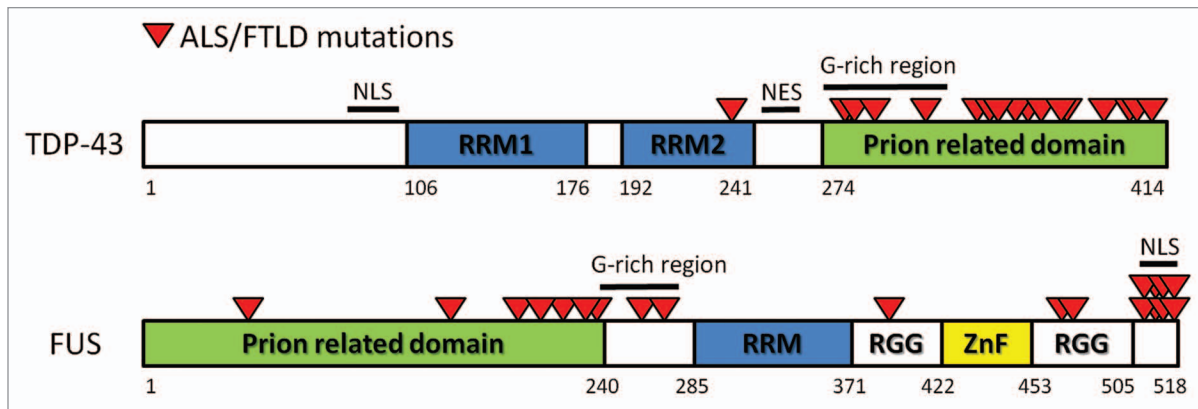
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\*Correspondence to: Robert H. Baloh;  
Email: rbaloh@wustl.edu

it typically has a focal site of onset in the nervous system, i.e., begins with unilateral hand weakness. Second, progression is characterized by apparent “spread” of neurodegeneration, usually to the contralateral hand, followed by involvement of the legs. Recent detailed autopsy studies of ALS patients have confirmed that loss of motor neurons is most pronounced at the site of onset and diminishes in a gradient fashion with further distance from that site.<sup>3</sup> While many aberrant phenomena including excitotoxicity, oxidative stress, mitochondrial dysfunction and altered axonal transport have been implicated in ALS pathogenesis, it is not easily apparent how any of these could explain the focal initiation or the progressive spread of the disease through the motor system.<sup>4</sup>

While the majority of ALS occurs sporadically, approximately 5–10% of patients have a family history of the disorder, typically autosomal dominant. For nearly 15 years the only known ALS gene was SOD1, mutations in which are responsible for ~20% of familial cases. In 2006, accumulations of a RNA binding protein called TDP-43 were identified in degenerating neurons in both ALS and the clinically overlapping disorder frontotemporal lobar degeneration (FTLD).<sup>5</sup> This was followed quickly by the identification of point mutations in TDP-43 in patients with familial ALS, indicating that altered TDP-43 function can be a primary cause of the disease.<sup>6–10</sup> Shortly thereafter mutations in a second RNA binding protein called FUS were reported in familial ALS.<sup>11,12</sup> Both TDP-43 and FUS are predominantly nuclear proteins involved in diverse aspects of RNA metabolism; however, in disease tissue both were observed to form inclusions in the cytosol

First described in 1869, amyotrophic lateral sclerosis (ALS or Lou Gehrig disease) is one of the longest known neurodegenerative diseases.<sup>1</sup> The clinical presentation typically involves progressive weakness and muscle atrophy (due to degeneration of spinal motor neurons) and spasticity and reflex disinhibition (due to degeneration of upper motor neurons in the motor cortex) with death from respiratory failure within 3–5 years. Since the earliest descriptions by both Charcot and Gowers,<sup>2</sup> ALS progression was understood to have several key features. First is that



**Figure 1.** Line diagrams of TDP-43 and FUS showing the relationship between the prion-related domains and mutations in ALS and FTLD. The location of the prion-related domains are based on experimental findings of their interactions with polyglutamine inclusions<sup>13,14</sup> and a prediction algorithm based on yeast prion domains.<sup>15</sup> In the case of TDP-43, all but one of the ALS associated mutations are located in the prion-related Q/N rich domain. In FUS, the majority of ALS associated mutations occur in the C-terminal nuclear localization signal (NLS). However, a second cluster also occurs in or adjacent to the N-terminal prion related domain. NES, nuclear export signal; RRM, RNA binding domain; RGG, arginine, glycine, glycine repeat rich region; ZnF, zinc finger domain.

of affected neurons. These findings suggested that aberrant protein aggregation may play a key role in ALS pathogenesis, akin to the central role of protein misfolding and aggregation observed in other neurodegenerative diseases. Interestingly, both FUS and TDP-43 contain “prion-related” Q/N rich domains and, in the case of TDP-43, essentially all of the ALS/FTLD associated mutations occur within this domain (Fig. 1).<sup>13-15</sup> Although the importance of the prion-related domains in FUS and TDP-43 remains unclear, investigation into their role in the normal and pathologic functions of the proteins clearly warrants attention and is the focus of this review.

### Prions and Prion-Related Domains

Prion protein remains the only known example of a protein capable of propagating a self-replicating conformation that can spread a disease (transmissible spongiform encephalopathy) across individuals and is thereby fitting of its name as an “infectious protein.”<sup>16,17</sup> However, additional proteins exhibiting prion-like behavior are also observed in yeast, invertebrate and mammalian cells. In these cases the adoption of an alternate protein conformation and template based spreading of this conformation to the normal form, appears not to be deleterious and cause disease, but instead regulates the function of the aggregating protein.

Prion-like behavior of proteins is best characterized in yeast.<sup>18,19</sup> The Sup35 protein is normally required for translational termination; however, under certain (particularly stressful) conditions it can form a self-propagating amyloid conformation transmissible to offspring, which is dependent on an intrinsically disordered region at the N-terminus particularly rich in glutamine (Q) and asparagine (N) residues.<sup>20</sup> Because this Q/N rich region is required for prion like propagation, it is referred to as the “prion domain.” Evidence supports that under stressful environmental conditions, induction of the Sup35 prion state leads to loss of Sup35 function and widespread read through of stop codons, allowing the rapid emergence of novel phenotypes.<sup>21</sup> Therefore, rather than representing a disease, prion domain mediated aggregation of Sup35 may actually be an adaptive strategy to provide immediate phenotypic diversity under stressful conditions.<sup>19</sup>

The prion-domains of most yeast prions are similarly Q/N rich, including those in Ure2 and Rnq1, although others (including HET-s) are not. Therefore, while Q/N rich domains are permissive to allow a protein to adopt a prion-like conformational state, they are not absolutely required. A growing body of work has supported that while not all Q/N domain containing yeast proteins can function as prions, they share a strong tendency to self-aggregate when overexpressed.<sup>22,23</sup>

There is also evidence for prion like behavior of a Q/N rich protein in *Aplysia*.<sup>24</sup> CPEB is a RNA binding protein involved in regulating local synaptic protein synthesis. Synaptic activity appears to shift apCPEB from a monomeric to a multimeric form, which is dependent on the Q/N rich domain. In the multimeric form, apCPEB is active and regulates local mRNA translation to maintain synaptic facilitation. Similar behavior has also been observed in *Drosophila* where the prion-related Q/N domain of Pumilio, another RNA binding protein, regulates self-aggregation and post-synaptic translational suppression.<sup>25</sup>

Finally, the mammalian genome contains a large number of proteins with Q/N rich prion related domains that may similarly use self-aggregation to modulate their activity.<sup>26</sup> A well studied example is the RNA-binding protein TIA-1, which is a key component of stress granules, cytoplasmic RNA-protein complexes formed under conditions of cellular stress which mediate mRNA translational suppression.<sup>27</sup> The prion related domain of TIA-1 is necessary for it to aggregate and organize stress granule formation.<sup>28</sup> A similar mechanism using Q/N domain mediated aggregation of RNA binding proteins also appears to be involved in the formation of P-bodies.<sup>29</sup>

Therefore, a consistent theme for proteins containing prion-related Q/N rich domains from yeast through mammals is

one of stimulus induced conformational change leading to self aggregation (often from environmental stress), which then alters protein function to organize an adaptive response (form stress granules, alter synaptic translation, etc.).

### **Evidence for Prion-Related Q/N Domains in TDP-43 and FUS**

Given that prion-related domains are inherently disordered in structure, with minimal primary sequence determinants other than enrichment of Q/N residues, the presence of these domains was not immediately apparent in TDP-43 and FUS. TDP-43 structurally resembles heterogeneous nuclear ribonuclear proteins (hnRNPs), and it was originally noted to have a glycine-rich domain (residues 274–314) similar to other “RBD-Gly” family proteins, including hnRNPA1.<sup>30,31</sup> Subsequently, some have referred to the entire C-terminal region as a glycine-rich domain analogous to hnRNPA1.<sup>32</sup> However, unlike hnRNPA1, the C-terminal domain of TDP-43 is not involved in binding to nucleic acids or nuclear shuttling.<sup>31,33,34</sup> Instead, the C-terminus of TDP-43 is required for it to function as a suppressor at several splicing targets<sup>35,36</sup> and for TDP-43 to act as a transcriptional insulator for the mouse sp-10 gene.<sup>37</sup> Importantly, all but one of the ALS associated mutations in TDP-43 occur in the C-terminal domain.

In an effort to define cellular stressors that regulate TDP-43 translocation from the nucleus, our group expressed several aggregation prone proteins in the cytosol of cultured cells, to determine if TDP-43 could act as a sensor for misfolded protein stress.<sup>14</sup> We observed that TDP-43 became tightly sequestered into detergent insoluble inclusions formed by polyglutamine proteins (Huntingtin N-terminal fragment or pure expanded polyglutamine), which required a particularly Q/N rich stretch of residues (31%) within the C-terminal domain of TDP-43. This region is similar in Q/N content to other proteins that were identified in unbiased screens for polyglutamine aggregate interacting proteins, including NF-Y, TIA-1 and FUS.<sup>13,38,39</sup> The presumed molecular basis of this interaction is the incorporation of the

Q/N rich domain into the fibrillar  $\beta$ -sheet structure of the polyglutamine inclusion. This provided the first experimental evidence that the C-terminus of TDP-43 behaves similarly to other proteins with Q/N rich prion-related domains.

Inclusions of either TDP-43 or FUS are observed in cases of ALS and FTLD. For TDP-43, the C-terminal region is highly prone to aggregation, both as purified protein *in vitro*<sup>40</sup> or when expressed as a fragment in yeast or cultured mammalian cells.<sup>41–43</sup> This strong tendency of the C-terminus of TDP-43 to self-associate and form aggregates is likewise consistent with the behavior of a prion-related Q/N domain containing protein.

Finally, several algorithms have been used to predict proteins that contain prion-related domains in both yeast and human genomes.<sup>22,23,26</sup> Most recently using a hidden Markov Model algorithm trained on known yeast prion domain containing proteins, FUS and TDP-43 were predicted as the fifteenth and sixtieth most likely to contain prion related domains out of nearly 30,000 proteins in the human genome.<sup>15</sup>

Therefore there are currently three pieces of evidence that TDP-43 and FUS contain prion-related domains: (1) both have modular domains highly enriched in Q/N residues that meet prediction criteria for prion-related domains; (2) both have a strong tendency to self-associate and form aggregates and (3) both are effectively cross-seeded into polyglutamine inclusions, mediated by the Q/N rich region similar to other prion-related domain containing proteins like TIA-1.

### **Implications of Prion-Related Domains in the Normal Function of TDP-43 and FUS**

As discussed above, a shared property of many proteins with prion-related Q/N domains is stimulus induced self aggregation, which alters the function of the protein in a switch like fashion. Therefore, a key question raised is whether TDP-43 and FUS undergo similar self aggregation in response to some type of stimulus and mediate a cellular response, akin to other Q/N rich domain containing proteins such as TIA-1. Both FUS and TDP-43

can be found in stress granules in cultured cells under certain conditions.<sup>44–49</sup> However, neither TDP-43 nor FUS appear to be required for stress granule formation, and FUS is only present in these structures if it is mislocalized to the cytosol by mutations in the nuclear localization signal.<sup>45,47</sup> Therefore it remains to be defined what role they actually play in stress granule mediated translational suppression. It is also important to point out that the same Q/N rich region of TDP-43 has been proposed to mediate protein-protein interactions with other components of the mRNA splicing machinery.<sup>50</sup> Therefore, the prion-related Q/N rich domain of TDP-43 may normally be involved in mediating protein-protein interactions for splicing regulation, and only in the disease state does it develop an alternate conformation that templates and seeds additional TDP-43 aggregation.

### **Implications of the Prion-Related Domains in TDP-43 and FUS in Neurodegeneration**

It is important to consider that TDP-43 “pathology” (cytoplasmic TDP-43 inclusions, nuclear clearing) is not only observed in ALS and FTLD but is also frequently present in affected brain regions in Alzheimer disease, Parkinson disease, chronic traumatic encephalopathy and even inclusion body myopathies.<sup>51–54</sup> This is quite consistent with the possibility that TDP-43 aggregation is part of a normal response to cellular stress, and is mediated by the prion-related domain. The TDP-43 inclusions themselves therefore may not be toxic or even protective, but instead are indicative of a stress response pathway involving TDP-43 that is activated in these cells. Therefore, defining a potential normal role of prion-domain mediated TDP-43 aggregation could help to explain the presence of TDP-43 aggregates in a wide variety of neurodegenerative conditions.

Another interesting implication of the presence of prion related Q/N domains in both TDP-43 and FUS is the known property of proteins with these domains to co-aggregate into inclusions of polyglutamine containing proteins.<sup>13,14</sup> This observation provided the initial experimental

evidence that TDP-43 contained a Q/N rich domain. Furthermore, sequestration of TDP-43 into polyglutamine inclusions led to a secondary loss of TDP-43 splicing regulation, and overexpression of TDP-43 rescued the toxicity of an N-terminal huntingtin fragment containing a polyglutamine expansion in cultured cells.<sup>14</sup> These findings are consistent with a model where TDP-43 and other Q/N rich proteins cross-seed with polyglutamine inclusions, which may be a mechanism of polyglutamine toxicity.<sup>39</sup> Recently, intermediate length polyglutamine expansions in Ataxin-2 were found to be associated with increased risk of ALS.<sup>55</sup> Although the details remain to be worked out, these findings further support the suggestion that there may be significant cross-talk between pathways of neurodegeneration in both polyglutamine diseases and ALS/FTLD, potentially mediated by the Q/N rich domains in TDP-43 and FUS.

Finally, the prion-related Q/N domains in TDP-43 and FUS have potential implications for the apparent “spread” of neurodegeneration throughout the motor system in ALS. Recently, increased attention has been focused on the concept that transmission of misfolded proteins involved in neurodegeneration (such as amyloid- $\beta$  or tau) could be propagated from cell to cell in a prion-like fashion.<sup>15,56,57</sup> Although experimental evidence for this hypothesis is nascent at present, it is attractive as a potential explanation for the clinically observed spread of neurodegenerative diseases throughout particular neuronal networks. Given that prion-related Q/N domains are capable of developing altered conformers which recruit aggregation of the native protein, the presence of prion-related domains in TDP-43 and FUS provides a potential molecular substrate for transmission of aggregates of these proteins from cell to cell.

## Conclusions

The discovery that TDP-43 and FUS play a key role in the pathogenesis of ALS and FTLN has been a significant breakthrough in understanding these diseases. It has emphasized the central role for protein misfolding, a common theme to most neurodegenerative diseases, and opened

investigations into the role of altered RNA metabolism, which was previously unexplored. Although an intriguing finding, additional studies are clearly needed to delineate the importance of the prion-related Q/N domains in TDP-43 and FUS. These include determining what role they play in normal protein function, under what conditions they mediate pathologic aggregation of TDP-43 and FUS, and whether they might play a role in disease progression by allowing cell to cell transfer of pathologic protein aggregates.

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