“Deciphering the amyloid foldome of TDP-43”

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ABSTRACT

TDP-43 is an essential regulator of RNA splicing and metabolism and its aggregates play key roles in devastating diseases, including Amyotrophic Lateral Sclerosis (ALS)¹, Frontotemporal Dementia (FTD) and Limbic-Predominant Age-Related TDP-43 Encephalopathy (LATE)². Besides this pathological aggregation, TDP-43's oligomerization also serves vital functions³, which adds urgency to determine pathological conformations of TDP-43. The recently published cryo-EM study by Cao, Eisenberg and coworkers now reveals amyloid structures of putative pathological aggregates from TDP-43's C-terminal region⁴. Whereas the Cao *et al.*'s cryo-EM structures contain both the hydrophobic and Q/N-rich segments, the data were interpreted mainly through the lens of hydrophobic contacts. However, the Q/N-rich region can form amyloid on its own^{5,6} and therefore additional considerations of the Q/N-rich segment's contributions will advance our understanding of TDP-43 aggregation.

TDP-43 contains three well-folded domains (residues 1-78, 104-176, and 192-262), and an intrinsically disordered, C-terminal region (residues 265 - 414) (Fig. 1a) containing two evolutionarily conserved segments, suggesting functional importance. These are a stretch of hydrophobic residues (321-340, colored **green** in the sequence shown below), followed by a Q/N-rich, prion-like segment (341-363, in **red**) punctuated by residues with a high turn-forming propensity (underscored):

MNFGAFSINP **AMMAAAQAAL** **QSSWGMMGML** AS**QQNQ**SGPS **GNNQNQ**GNMQR EPN**Q**
 311 321 331 341 351 361

Cao *et al.* present the first cryo-EM structures of three polymorphic amyloid fibrils formed by this conserved region of TDP-43, which they called “SegA”. In all three polymorphs (named *sym*, *asym*, and *slow*), residues within the hydrophobic stretch (321-340) adopt an essentially identical “dagger-shaped” fold (Fig. 1b). Conversely, residues within the contiguous Q/N-rich region (341-360) adopt a variety of conformations, with residues 341-347 being solvent exposed in the two polymorphic structures regarded as “pathologically relevant” (SegA-*sym* and SegA-*asym*) (Fig. 1b). This contrasts previous results by Hasegawa and coworkers, who characterized TDP-43 fibrils derived from two post mortem ALS patient brains using mass spectrometry⁷, and found that Q and N residues within the Q/N-rich stretch, and especially within the 341-348 stretch, were scarcely modified in both brain samples (Fig. 1a). This points to these residues being protected inside the fibrillar core in pathologically relevant, *in vivo* TDP-43 aggregates, which is consistent with our structural model of the TDP-43 amyloid, formed by β -hairpins composed of residues 341-357 assembling with a β -turn configuration^{5,6}.

Cao *et al.* also reported the structure of a third polymorph, SegA-*slow*. Unlike SegA-*sym* and SegA-*asym*, the entire Q/N-rich segment is visible in SegA-*slow*, and therefore it could have been compared to the structural model for the Q/N-rich region advanced by Mompeán *et al.*⁶. In particular, the Q/N-rich segment in SegA-*slow* adopts β -hairpin conformation, where residues 341-348 are buried in the fibril core (Fig. 1c), in agreement with the data from brain-derived pathological aggregates discussed above⁷, and contrasting the conformations in SegA-*sym* and SegA-*asym*.

The β -hairpin in the SegA-*slow* structural model has a β -arc configuration, where each of the two β -strands contributes to two different β -sheets (Fig. 1c). By contrast, in our model, β -hairpins are in the β -turn configuration, and the two β -strands contribute to the same β -sheet (Fig. 1c). Interestingly, our previous results indicate that β -arc hairpins formed by the Q/N-rich segment cannot seed the growth of amyloids, whereas the β -hairpin with a β -turn configuration can⁶ (Fig. 1d). Perhaps in the SegA construct, the presence of the 311-320 nonpolar segment (in blue, Fig. 1a) confers the flexibility necessary for a preferential hydrophobic-driven assembly (i.e., initial formation of the dagger-shaped structure by residues 321-340), which limits the conformations that the remaining Q/N-rich segment (residues 341-360) may adopt (note that the Q/N-rich region is pruned right at the edge in SegA, Fig. 1a). In this regard, we note that β -arc hairpins are commonly seen in predominantly hydrophobic amyloids, whereas Q-rich fibrils feature hairpins with β -turns⁸. According to the study by Hasegawa and coworkers cited earlier⁷, the M, S and Q+N residues of the hydrophobic stretch that form the dagger-shaped structure in SegA were oxidized, phosphorylated and deamidated, respectively, in both ALS brain samples. This suggests that they are relatively exposed in bona fide TDP-43 fibrils *in vivo* (Fig. 1a), and that Q/N-driven amyloids may be more relevant. There is additional evidence pointing to the ability of the Q/N-rich segment to drive the assembly:

- 1) TDP-43 constructs with repeats of the Q/N-rich segment are able to induce and reproduce ALS pathological hallmarks in cells⁹.
- 2) H-bond hypercooperativity among aligned Q/N side chain amide groups are exceptionally strong and decisively stabilize Q/N-rich amyloid structures, including TDP-43, with respect to their hydrophobic counterparts^{6,10}.
- 3) Distinct aggregates of TDP-43 from different pathological tissues are reminiscent of prion strains¹¹. In this regard, TDP-43 remains functional when replacing its C-terminal domain with a Q/N-rich prion domain¹².
- 4) TDP-43 assembles into a functional amyloid in myogranules³, which displays a fibrillar morphology which closely resembles that of the amyloid with the β -turn β -hairpin conformation formed by the Q/N-rich region⁶. Both are recognized by the A11 antibody.

Finally, in addition to forming homo-oligomeric assemblies, TDP-43 interacts with Ataxin-2¹³ and polyQ segments through its C-terminal region¹⁴, and also recruits Nucleoporins (Nups) into TDP-43+Nups co-aggregates in disease¹⁵. These interactions among Q/N-rich domains strongly suggest that hybrid amyloids are formed, which could resemble the recently determined structure of the RIPK1-RIPK3 human necrosome, where Q residues are key to RIPK1-RIPK3 assembly¹⁶. The structures presented here by Cao *et al.* undoubtedly reveal an important portion of the TDP-43 aggregate foldome, highlighting the role of the hydrophobic segment. Nonetheless, based on the considerations mentioned here, the role of the Q/N-rich region in pathologically relevant structures might well be more central.

Fig. 1 | Q/N-rich and hydrophobic segments in TDP-43 aggregation. **a**, Structural domains of TDP-43: N-terminal domain (NTD, in yellow); RNA-recognition motifs (RRMs, in brown); C-terminal domain (in blue), which contains the hydrophobic (green) and Q/N-rich (red) regions that form amyloids with the topologies indicated. Linker regions are shown in gray. The region 311-360 is expanded, as the SegA construct contains these residues, with the pattern of oxidations (Met), phosphorylations (Ser) and deamidations (Gln & Asn) seen by Hasegawa and co-workers⁷ indicated as follows: / = modified in brain 1, \ = modified in brain 2, X = modified in both brains. **b**, Residues within the hydrophobic stretch (321-340 in green) adopt an essentially identical “dagger-shaped” fold in SegA-sym (top), SegA-asm (middle). Residues within the Q/N-rich region (in red) adopt different conformations. The bottom figure illustrates the overlap between SegA-sym, SegA-asm and SegA-slow. **c**, Left: the dagger-shaped fold in SegA-slow is preserved (green), and the Q/N-rich region (red) adopts a hairpin conformation with a β -arc configuration. Middle: each β -strand of the β -arc hairpin contributes to a different β -sheet. Right: the Q/N-rich region adopts a hairpin conformation with a β -turn configuration, where the two β -strands contribute to the same β -sheet. **d**, Hairpins with the β -turn configuration can assemble amyloids (left path), whereas β -arc can not (right path).

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Author contributions

M.M., E.B., and D.V.L. edited and co-wrote the manuscript.

Competing interest

The authors declare no competing interests.

References

1. Neumann, M. et al. Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. *Science* **314**, 130–633 (2006).
2. Nelson, P. T. et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. *Brain*. **146**(6), 1503–1627(2019).
3. Vogler, T. O. et al. TDP-43 and RNA form amyloid-like myo-granules in regenerating muscle. *Nature* **563**, 508–513 (2018).
4. Cao, Q., Boyer, D. R., Sawaya, M. R., Ge, P. & Eisenberg, D. S. Cryo-EM structures of four polymorphic TDP-43 amyloid cores. *Nat. Struct. Mol. Biol.* **26**, 619–627 (2019).
5. Mompeán, M. et al. Structural characterization of the minimal segment of TDP-43 competent for aggregation. *Arch. Biochem. Biophys.* **545**, 53–62 (2014).
6. Mompeán, M. et al. Structural evidence of amyloid fibril formation in the putative aggregation domain of TDP-43. *J. Phys. Chem. Lett.* **6**, 2608–2615 (2015).
7. Kametani, F. et al. Mass spectrometric analysis of accumulated TDP-43 in amyotrophic lateral sclerosis brains. *Sci. Rep.* **6**, 23281 (2016).
8. Hoop, C. D. et al. Huntingtin exon 1 fibrils feature an interdigitated β -hairpin-based polyglutamine core. *Proc. Natl. Acad. Sci.* **113**, 1546–1551 (2016).
9. Budini, M. et al. Cellular model of TAR DNA-binding protein 43 (TDP-43) aggregation based on its C-terminal Gln/Asn-rich region. *J. Biol. Chem.* **287**, 7512–7525 (2012).
10. Mompeán, M., Nogales, A., Ezquerra, T. A., & Laurents, D. V. Complex system assembly underlies a two-tiered model of highly delocalized electrons. *J. Phys. Chem. Lett.* **6**, 1831–1864 (2016).
11. Laferrière, F. et al. TDP-43 extracted from frontotemporal lobar degeneration subject brains displays distinct aggregate assemblies and neurotoxic effects reflecting disease progression rates. *Nat. Neurosci.* **22**, 65 (2019).
12. Wang, I. F. et al. The self-interaction of native TDP-43 C terminus inhibits its degradation and contributes to early proteinopathies. *Nat. Commun.* **3**, 766 (2012).
13. Becker, L. A. et al. Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. *Nature* **544**, 367–371 (2017).
14. Fuentealba, R. A. et al. Interaction with polyglutamine aggregates reveals a Q/N-rich domain in TDP-43. *J. Biol. Chem.* **285**, 26304–26314 (2010).

15. Chou, C. C. et al. TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. *Nat. Neurosci.* **21**, 228–239 (2018).
16. Mompeán, M. et al. The structure of the necrosome RIPK1-RIPK3 core, a human hetero-amyloid signaling complex. *Cell* **173**, 1244–1253 (2018).