https://doi.org/10.1038/d41586-021-03605-0

News & views

Neurodegeneration

Aggregates of TDP-43 protein spiral into view

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In some neurodegenerative diseases, a protein called TDP-43 forms aggregates in the brain, resulting in neuronal cell death. The structure of these aggregates and their properties have been unveiled.

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are two devastating neurodegenerative diseases that share disease mechanisms and underlying genetics, and can co-occur in the same individual as ALS–FTLD. ALS is commonly characterized by the deterioration of the motor neurons that control movement, whereas FTLD is associated with the deterioration of neurons in a part of the brain called the cortex, leading to behavioural changes and memory loss. Unfortunately, there are no effective therapies for ALS and FTLD, and early diagnosis is challenging. A common hallmark of both conditions is the abnormal accumulation of a protein called TDP-43 inside degenerating neurons[1–3]. Writing in *Nature*, Arseni *et al.*[4] report the structure of TDP-43 aggregates isolated from the brains of two individuals who had died from ALS–FTLD.

TDP-43 is a crucial RNA-binding protein, and is situated mainly in the cell nucleus, where it regulates the expression of many genes[3,5]. Attempts to understand the mechanisms that underlie the mislocalization and aggregation of TDP-43 in the cytoplasm are underway[3]. However, a major barrier limiting the development of diagnostic and therapeutic agents for ALS and FTLD is that the structure of TDP-43 aggregates in the brain has remained unknown.

Arseni and colleagues extracted filaments made of aggregated TDP-43 from two brain regions, the frontal cortex and the motor cortex – not an easy feat. First, they confirmed the disease-associated characteristics of the isolated TDP-43 aggregates. These characteristics[1,2] included insolubility, unusually extensive addition of phosphate groups (phosphorylation) to the serine amino-acid residues at positions 409 and 410 of the TDP-43 protein, and the presence of carboxy-terminal fragments of TDP-43.

Next, using a technique called cryo-electron microscopy (cryo-EM), Arseni *et al.* showed that TDP-43 molecules stack on top of one another, separated by about 4.8 ångströms, to form a single protofilament with a right-handed helical twist of approximately 1.4°. Intriguingly, cryo-EM also revealed that, perpendicular to the helical axis, TDP-43 forms what the authors call a double-spiral fold (Fig. 1).

This double-spiral fold is formed by 79 amino-acid residues of TDP-43, extending from position 282 (a glycine) to position 360 (a glutamine). This region lies in a domain of TDP-43 that has been described as 'prion-like' because of its similarity to another domain type that enables various yeast proteins to form what are known as prions[3]. These are infectious proteins that encode heritable traits in yeast. It is the prion-like domain that renders TDP-43 intrinsically prone to aggregation[3,6].

The double-spiral fold comprises three segments of the TDP-43 prion-like domain: a region at the amino-terminal end that is rich in the amino acid glycine; a hydrophobic region that forms the nucleus of the fold; and the

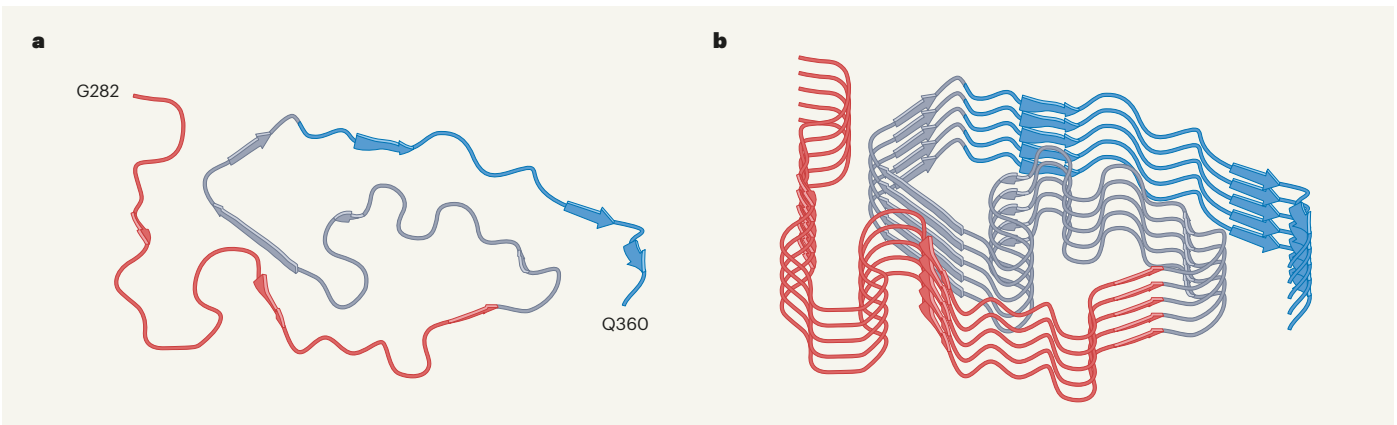

Figure 1 | TDP-43 filaments from individuals with ALS–FTLD adopt a double-spiral fold. Arseni *et al.*[4] used a technique called cryo-electron microscopy to determine the structure of filaments formed by the protein TDP-43 that were isolated from the brains of two individuals who had died from a combination of two disorders of the nervous system: amyotrophic lateral sclerosis with frontotemporal lobar degeneration (ALS–FTLD). The filaments are formed by stacks of individual TDP-43 molecules. **a**, The structure of the portion of the protein that was stacked, from the glycine residue at position 282 (G282) in the amino-acid sequence of TDP-43 to the glutamine at position 360 (Q360), forms a double-spiral fold. This fold consists of three major regions: a glycine-rich region (amino acids 282–310; red); a hydrophobic region (amino acids 311–342; grey); and a region rich in the amino acids glutamine and asparagine (343–360; blue). Arrows represent structural elements called β-strands. **b**, A filament consisting of five TDP-43 molecules is shown. (Adapted from ref. 4, Extended Data Figs 4b and 6b.)

C-terminal end that is rich in the amino acids glutamine and asparagine (Fig. 1). Notably, the hydrophobic region enables TDP-43 to undergo a process called liquid–liquid phase separation, whereby the protein molecules condense to form membrane-less organelles[3,5]. This finding indicates that disease-associated TDP-43 filaments might originate from such liquid compartments[3].

The presence of the double-spiral fold of TDP-43 filaments raises several exciting questions. How does this fold affect modifications of TDP-43 that occur after the protein chain is first synthesized? Which surfaces of the TDP-43 filaments are likely to capture other macromolecules? And how does the TDP-43 filament structure compare with those of protein filaments that accumulate in other neurodegenerative diseases?

Examining known phosphorylation sites associated with TDP-43 aggregation, Arseni and co-workers noted that the sites that are abnormally phosphorylated in ALS–FTLD (the serine residues at positions 409 and 410) lie outside the fold and are therefore accessible and compatible with the structure. That is, these phosphorylation events could occur after TDP-43 has aggregated. Furthermore, the authors characterized the TDP-43-filament surface and discerned two distinct features: a prominent groove formed by the main chain of amino acids 282–286 at the N-terminal end, and polar patches that arise from the abundance of glutamine and asparagine residues at the C-terminal end. These two features probably enable TDP-43 filaments to engage with various macromolecules.

Notably, these surface features are not found in disease-associated filaments made of other proteins, such as the tau filaments and α-synuclein filaments that are seen in the brains of people with Alzheimer's disease and Parkinson's disease, respectively[7,8]. On the basis of these findings, the authors suggest that the macromolecules that interact with TDP-43 aggregates are probably distinct from those that interact with protein filaments associated with other neurodegenerative diseases. This would hint that the mechanisms underlying ALS and FTLD differ from those associated with these other disorders.

Another crucial aspect to consider concerns ALS-associated mutations in the gene encoding TDP-43, which can sometimes alter the region of the protein that forms the double-spiral fold. Would these alterations have any effect on the double-spiral-fold structure? Arseni *et al.* report that, of 24 mutations that affect that region of TDP-43 and that are associated with ALS, 18 are predicted to result in TDP-43 variants that could form the double-spiral-fold structure. Thus, it will be enlightening to establish whether TDP-43 filaments adopt different folds in individuals who carry the mutations that are not compatible with the double-spiral-fold structure.

Finally, stark differences were revealed between the structures of brain-derived TDP-43 filaments and synthetic filaments assembled from portions of the TDP-43 prion-like domain[9,10]. The inconsistencies between these structures highlight the difficulty in developing models of ALS–FTLD *in vitro* that faithfully recapitulate the disease. A major challenge will be to generate the double-spiral fold with synthetic TDP-43. Furthermore, in developing and regenerating muscle, TDP-43 forms filaments that might be required in proper muscle formation[11]. However, whether these TDP-43 filaments resemble the TDP-43 filaments found in neurons of individuals with ALS–FTLD remains unclear.

Unveiling the structure of TDP-43 filaments is a milestone that opens exciting avenues for the development of new diagnostic and therapeutic strategies. One challenge with ALS–FTLD is that there are no tools for early diagnosis, and no dye compounds are available that bind to TDP-43 filaments in the brain to image them. The double-spiral-fold structure revealed by Arseni and colleagues might enable the design of imaging dyes. It will also be of interest to determine how therapeutic enzymes that break up protein aggregates might act on the double-spiral fold of TDP-43 filaments to make the protein soluble[3]. Excitingly, the structure should help to inform the development of small-molecule drugs that prevent the formation of the double-spiral fold and inhibit TDP-43 aggregation. Such drugs could provide an urgently needed intervention for treating these debilitating disorders.

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J.S. declares competing interests. See go.nature.com/3dcfmrr for details.