News & views

Neurodegeneration

Mining protein fibrils using structural biology

Hideyuki Takahashi & Stephen M. Strittmatter

Protein fibrils accumulate in the brain during neurodegeneration. Cryo-electron microscopy has now uncovered fibrils of a protein not previously thought to accumulate. **See p.304 & p.310**

Neurodegenerative diseases often involve the formation of insoluble protein aggregates called fibrils in the brain, with specific proteins characterizing different diseases. For example, amyloid-β fibrils form in Alzheimer's disease, whereas α-synuclein fibrils define Parkinson's disease. The study of these fibrils tends to follow a set path: the discovery of an aggregate in brain tissue analysed at autopsy, then analysis of its biochemistry; genetic research to pinpoint mutations associated with the abnormal protein; functional studies to investigate how the protein is altered in disease; and, finally, structural biology of the disease-related protein fibril. Schweighauser *et al.*[1] (page 310) and Jiang *et al.*[2] (page 304) turn this discovery pathway on its head. In the process of using cryo-electron microscopy at near-atomic resolution to investigate the structure of known aggregates, they have detected the presence of a previously unknown protein fibril in the brain.

The protein TDP-43 forms aggregates in most cases of two neurodegenerative diseases: a type of dementia called frontotemporal lobar degeneration (FTLD) and motor neuron disease (or amyotrophic lateral sclerosis, ALS)[3]. Several groups have been attempting to resolve structures of disease-related TDP-43 assemblies, with one team so far suceeding[4].

Trying to resolve TDP-43 structures, Schweighauser *et al.* and Jiang *et al.* (together with Chang *et al.*[5], whose study was recently published in *Cell*) discovered fibrillary material that could not be fitted to TDP-43 in the brains of people with FTLD who had died. In each case, models that predict amino-acid sequence from structural features indicated a match with the sequence of a protein called TMEM106B, but not with other proteins. Schweighauser and colleagues also found TMEM106B fibrils in the brains of people who had died with a range of other neurodegenerative diseases, including ALS, and even in healthy older individuals.

All three papers showed that the TMEM106B fibrils stack into protofilaments — single protofilaments form rod-like structures, and pairs of protofilaments form twisted ribbons. Each study found a similar structure for the TMEM106B fibrils, each of which was rich in secondary-structure elements called β-strands, comprising 17–19 such segments per fibril. Jiang and co-workers compare the structure to an 18-hole golf course, with the strands representing fairways of varying length and curvature.

Functional TMEM106B is a transmembrane protein involved in an intracellular endolysosomal pathway, through which molecules are transported in endosome structures from the cell's plasma membrane to organelles called lysosomes for degradation[6]. TMEM106B physically interacts with and modulates the activity of an enzyme called vacuolar ATPase, modifying the pH of the endolysosome (the structure that forms when the endosome and lysosome fuse together for the transfer of molecules)[7]. Schweighauser *et al.* and Jiang *et al.* show that TMEM106B fibrils are composed of the carboxy-terminal fragment of the TMEM106B protein — specifically amino-acid residues 120–254, which would usually be inside the endolysosome (Fig. 1). The current studies suggest that the cleavage between residues 119 and 120 is essential for fibril formation, because the cleaved terminus is buried in the fibril core, leaving no space for other residues. Previously, TMEM106B has been studied using antibodies that bind to other portions of the protein[8], providing a potential explanation for why TMEM106B fibrils have not previously been described.

The presence of TMEM106B fibrils in the brains of people with neurodegenerative diseases links to existing information about the protein. For instance, variation in the *TMEM106B* gene is a known risk factor for FTLD[9]. And a genome-wide association study has suggested that *TMEM106B* variation

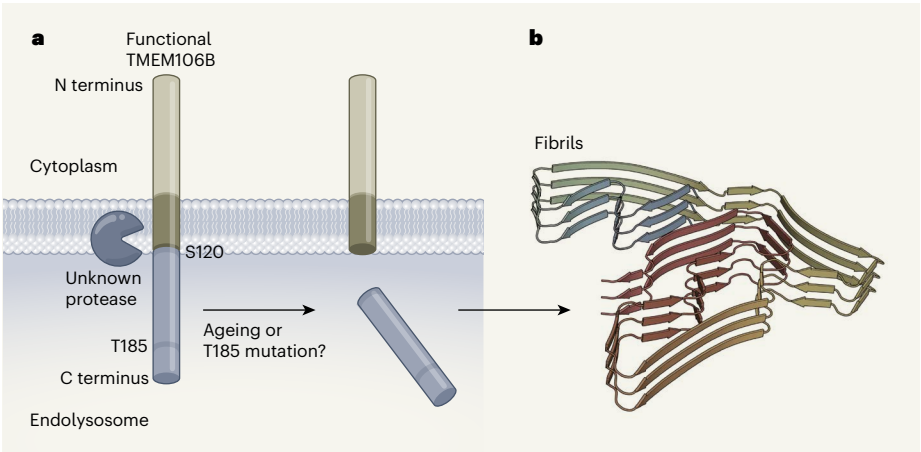

Figure 1 | Possible pathway for the formation of TMEM106B fibrils. Three groups[1,2,5] have identified insoluble fibrils of the protein TMEM106B in the brains of people with neurodegenerative diseases. One group[1] also found the fibrils in the brains of healthy older people. **a**, Functional TMEM106B spans the membrane of intracellular endolysosomal compartments in the cytoplasm. The formation of fibrils requires cleavage of the carboxy-terminal fragment of TMEM106B at amino-acid residue S120, by an unknown protease enzyme. This process might be modulated by ageing, or by genetic mutations that alter amino-acid residue T185. **b**, The C-terminal fragment forms a fibril that consists of multiple β-sheet strands of varying length and curvature (each indicated by a coloured arrow). Fibrils stack on top of each other to form protofilaments. (Part **b** of the figure adapted from Fig. 3 of ref. 2.)

increases Alzheimer's disease risk[10]. However, the detection of TMEM106B fibrils in a range of samples raises questions about their relevance to disease.

Do these fibrils cause disease, or are they ancillary and inconsequential? Schweighauser and colleagues suggest that the presence of these fibrils corresponds most specifically to age, regardless of whether or not a person has a neurodegenerative condition — a theory that is consistent with Chang and colleagues' observation of similar fibrils across several diseases. By contrast, Jiang and co-workers observed TMEM106B fibrils in people who had FTLD, but not in age-matched control individuals. None of the studies involves enough samples to have the statistical power to associate TMEM106B fibrils with a specific disease state. It is notable that, although the conclusions reached by each group are markedly different, the characterization of TMEM106B fibrils and the extraction methods they used to obtain the fibrils are strikingly similar.

Further work will be required to explain this discrepancy and determine whether the fibrils are harmful, irrelevant or even protective against disease. TMEM106B fibrils might not cause disease in their own right, but instead reflect mounting changes in the ageing brain, similar to the accumulation of lipofuscin — amorphous deposits of oxidized lipid and protein fragments, which are found in most ageing brains, regardless of disease status[11]. Even though it is found ubiquitously in ageing brains, the early accumulation of lipofuscin is associated with rare, inherited lysosomal disorders[12]. Given that the risk of developing a neurodegenerative disease increases with age, and that TMEM106B fibrils might accumulate with age, it is possible that the presence of TMEM106B fibrils enhances mechanisms of neurodegeneration driven by other factors and protein aggregates. Going forward, research priorities should include determining the prevalence of TMEM106B fibrils and their effect on endolysosomal cell biology and on neurodegenerative diseases that involve other types of fibril.

There are other avenues for investigation, too. First, what is the identity of the protease enzyme that cleaves transmembrane TMEM106B? If TMEM106B fibrils are harmful, inhibition of this protease might have therapeutic potential. Second, the three studies found fibrils derived from individuals who have a common, disease-protective variant of TMEM106B, as well as fibrils without that variant, indicating that the variant itself is not directly responsible for the propensity of TMEM106B to form fibrils. So how does the genetic association between TMEM106B and neurodegeneration fit into the picture? Third, the cryo-electron-microscopy structures reveal evidence of a small molecule bound to TMEM106B, the identity of which remains unknown. Determining the nature of this molecule will allow researchers to assess its potential role in facilitating fibril formation and in disease, as well as its relationship to components of lipofuscin.

Finally, TDP-43 fibrils were not observed in these studies. This could imply that the bulk of aggregated TDP-43 formed in neurodegeneration does not adopt a regular fibril structure of the sort described recently[4]. Alternatively, perhaps TDP-43 fibrils cannot be extracted reliably or are unstable under the standard tissue-processing methods used in the current papers.

Structural biology has provided unexpected insights into the fibrils that accumulate in ageing and diseased brains. Researchers must now backtrack along the typical path for analysis of protein aggregates to glean insights into the epidemiology and biochemistry of TMEM106B so we can understand its mechanism of action.

Hideyuki Takahashi and **Stephen M. Strittmatter** are in the Departments of Neuroscience and Neurology, Kavli Institute for Neuroscience, Yale School of Medicine, New Haven, Connecticut 06510, USA.
e-mail: stephen.strittmatter@yale.edu

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Cell biology

A rethink about enzymes that drive DNA replication

Masato T. Kanemaki

It has long been thought that two enzymes, the kinases CDC7 and CDK2, are both needed to trigger DNA replication in mammalian cells. This view is challenged by evidence that offers a revised view of which kinases are essential. **See p.357**

If you read the scientific literature about DNA replication, you will find that two complexes, each containing an enzyme called a kinase and a regulatory protein, are involved in the initiation of this process. On page 357, Suski *et al.*[1] provide evidence of the need for a re-evaluation of the kinases involved.

According to previous studies, the two complexes required for DNA replication contain the kinases CDC7 and CDK2, and are called, respectively, CDC7–DBF4 and CDK2–cyclin E (Fig. 1a). These complexes[2,3] drive DNA replication at the stage of the cell cycle called S phase. They do this by promoting conversion of the inactive enzyme complex MCM2–MCM7 to an active complex that unwinds double-stranded DNA to form a large protein complex called the replisome — which synthesizes DNA — and by modifying proteins called replication factors.

However, our understanding of this process has come mainly from studies of yeast and from a cell-free system that uses egg extracts from the frog *Xenopus laevis*. The proteins involved in DNA replication are, in many cases, essential for cell proliferation, and so interfering with replication can kill the cells. Hence, when assessing the role of a target protein, it is necessary to evade such lethality. This can be achieved in yeast and in *X. laevis* using methods available to deplete or inactivate target proteins, but no comparably effective methods for doing this have been available in mammalian cells.

Suski and colleagues revisited the question of whether CDC7 is essential for DNA replication by using modern chemical-genetic techniques to produce versions of CDC7 that could be controllably depleted or inhibited. They used a system in which a version of CDC7 (containing what is called an auxin-inducible degron domain) is rapidly degraded by the molecule auxin[4]. Suski *et al.* also engineered a CDC7 that had an altered pocket for binding to the molecule ATP, so that the kinase's activity would be inhibited[5] by bulky molecules with structures similar to ATP.

These experiments unexpectedly revealed that CDC7 was dispensable for DNA replication not only in mouse and human cells grown *in vitro*, but also in mice. Furthermore, the authors found that the complex CDK1–cyclin B