Distinct Conformers of Assembled Tau in Alzheimer's and Pick's Diseases

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Tau filaments with distinct morphologies and/or isoform compositions underlie a large number of human neurodegenerative diseases. In conjunction with experimental studies, this has led to the suggestion that conformers of aggregated tau exist. Electron cryo-microscopy can be used to determine high-resolution structures of amyloid filaments from human brain. Paired helical and straight tau filaments of Alzheimer's disease (AD) are ultrastructural polymorphs. Each filament core is composed of two identical protofilaments extending from G273/304–E380 (in the numbering of the 441-amino acid isoform of human tau), which adopt a combined cross- β -helix structure. They comprise the ends of the first or second microtubule-binding repeat (R1 or R2), the whole of R3 and R4, and 12 amino acids after R4. In contrast, the core of the narrow filaments of Pick's disease (PiD) consists of a single protofilament extending from K254–F378 of 3R tau, which adopts a cross- β structure. It comprises the last 21 amino acids of R1, all of R3 and R4, and 10 amino acids after R4. Wide tau filaments of PiD, which are in the minority, consist of two narrow filaments packed against each other. The tau filament folds of AD and PiD appear to be conserved between different cases of disease. These findings show that filamentous tau adopts one fold in AD and a different fold in PiD, establishing the existence of distinct conformers.

The ordered assembly of fewer than 10 different proteins into filamentous assemblies defines the majority of cases of age-related neurodegenerative diseases, including Alzheimer's and Parkinson's. Most cases of disease are sporadic, but a small percentage is inherited in a dominant manner. Huntington's disease is an exception, because it is always inherited. Work performed over the past 35 years established a causative role for filament formation in inherited forms of disease. By extrapolation, it appears likely that inclusion formation is central to neurodegeneration in all sporadic cases of disease. Tauopathies, which are characterized by the assembly of tau protein, are the most common proteinopathies of the human nervous system. They include cases of Alzheimer's disease (AD), Pick's disease (PiD), chronic traumatic encephalopathy (CTE), tangle-only dementia, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease (AGD), and several rarer diseases.

TAU PROTEIN

Tau belongs to the family of intrinsically disordered proteins, which have many interaction partners and are often implicated in neurodegenerative diseases (Goedert et al. 2017). Tau can be divided into an amino-terminal domain, a proline-rich region, the repeat domain, and a carboxy-terminal domain. The amino-terminal domain projects away from the microtubule surface and is believed

to interact with components of the neuronal plasma membrane. An interaction between exon 1 and annexins may help to explain the axonal localization of tau (Gauthier-Kemper et al. 2018). Exon 1 contains a primate-specific sequence between residues 18 and 28 of human tau that has been proposed to mediate interactions with neuronal proteins (Stefanoska et al. 2018). The PXXP motifs in the proline-rich region are recognized by SH3 domain-containing proteins of the Src family of nonreceptor tyrosine kinases, such as Fyn (Lee et al. 1998).

The repeat domain and some adjacent sequences mediate interactions between tau and microtubules. Electron cryo-microscopy (cryo-EM) has shown that each tau repeat binds to the outer microtubule surface and adopts an extended structure along protofilaments, interacting with α - and β -tubulin (Al-Bassam et al. 2002; Kellogg et al. 2018). Single-molecule tracking revealed a kiss-and-hop mechanism, with a dwell time of tau on individual microtubules of ~40 msec (Janning et al. 2014; Niewidok et al. 2016). Isoform differences do not influence this interaction. Despite these rapid dynamics, tau promotes microtubule assembly. It remains to be seen if microtubules are also stabilized. Individual axonal microtubules have stable and labile domains. Tau is most abundant in the labile domain, which has led to the suggestion that it may not stabilize microtubules but enable them to have long labile domains (Black et al. 1996; Qiang et al. 2018). Less is known about the function of the carboxy-terminal region, which may inhibit assembly into filaments. Although it

lacks a typical low-complexity domain, full-length tau has been shown to undergo liquid–liquid phase separation, which has been reported to initiate aggregation, at least in vitro (Zhang et al. 2017b; Wegmann et al. 2018). Tau is subject to a large number of posttranslational modifications, including phosphorylation, acetylation, methylation, glycation, isomerization, O-GlcNAcylation, nitration, sumoylation, ubiquitination, and truncation (Goedert et al. 2017).

TAU ISOFORMS

Six tau isoforms ranging from 352 to 441 amino acids in length are expressed in adult human brain from a single *MAPT* gene (Fig. 1A; Goedert et al. 1989). They differ by the presence or absence of inserts of 29 and 58 amino acids (encoded by exons 2 and 3, with exon 3 being only transcribed in conjunction with exon 2) in the amino-terminal half, and the inclusion, or not, of the 31-amino acid microtubule-binding repeat, encoded by exon 10, in the carboxy-terminal half. Inclusion of exon 10 results

in the production of three isoforms with four repeats (4R) and its exclusion in a further three isoforms with three repeats (3R). The repeats comprise residues 244–368, in the numbering of the 441-amino acid isoform. In adult human brain, similar levels of 3R and 4R tau are expressed; the finding that a correct isoform ratio is essential for preventing neurodegeneration came as a surprise. 2N isoforms are underrepresented in comparison with isoforms that include exon 2 or exclude both exons 2 and 3; 2N, 1N, and 0N tau isoforms make up 9%, 54%, and 37%, respectively. Big tau, which carries an additional large exon in the amino-terminal half, is only expressed in the peripheral nervous system (Couchie et al. 1992; Goedert et al. 1992).

Tau isoform expression is not conserved between species. Thus, in adult mouse brain, 4R tau isoforms are almost exclusively present, whereas adult chicken brain expresses 3R, 4R, and 5R tau isoforms. However, the presence of one hyperphosphorylated 3R tau isoform lacking amino-terminal repeats is characteristic of developing vertebrates. In mice, the switch from 3R to 4R tau occurs between postnatal days 9 and 18, with tau phos-

Figure 1. Human brain tau isoforms and disease-causing *MAPT* mutations. (A) *MAPT* and the six tau isoforms expressed in adult human brain. *MAPT* consists of 14 exons (E). Alternative mRNA splicing of E2 (red), E3 (green), and E10 (yellow) gives rise to six tau isoforms (352–441 amino acids). The constitutively spliced exons (E1, E4, E5, E7, E9, E11, E12, and E13) are shown in gray. E6 and E8 (violet) are not transcribed in human brain. E4a (orange) is only expressed in the peripheral nervous system. The repeats (R1–R4) are shown, with three isoforms having four repeats (4R) and three isoforms with three repeats (3R). The core sequences of tau filaments from Alzheimer's disease (G273/305–E380) determined by cryo-EM are underlined (in orange); the core sequences of tau filaments from Pick's disease (K254–F378 of 3R tau) are also underlined (in blue). (B) Mutations in *MAPT* in FTDP-17T. Fifty coding region mutations and 10 intronic mutations flanking E10 are shown.

phorylation decreasing over time (Tuerde et al. 2018). However, isoform switching and phosphorylation are regulated differently. Adult 4R tau isoforms are better at promoting microtubule assembly and binding to microtubules than the 3R tau isoform expressed during development (Goedert and Jakes 1990). This is consistent with the need for a more dynamic cytoskeleton during the development of nerve cells.

TAU ASSEMBLIES

Full-length tau assembles into filaments; the repeats and some adjoining sequences form the filament core, with the amino-terminal half and the carboxyl terminus giving rise to the fuzzy coat (Goedert et al. 1988; Wischik et al. 1988a,b). Tau filaments from human brain and those assembled in vitro from overexpressed protein have a cross- β structure characteristic of amyloid (Berriman et al. 2003). Because the region that binds to microtubules also forms the core of tau filaments, physiological function and pathological assembly may be mutually exclusive.

Phosphorylation negatively regulates the ability of tau to interact with microtubules and filamentous tau is abnormally hyperphosphorylated (Iqbal et al. 2016). However, it remains to be proved that phosphorylation is the trigger for aggregation in human diseases. Alternatively, a change in conformation as part of aggregation may lead to tau hyperphosphorylation. Because tau is hydrophilic, it is not surprising that the unmodified full-length protein requires cofactors, such as heparin, to assemble into filaments (Goedert et al. 1996; Pérez et al. 1996). Cofactors other than heparin and/or posttranslational modifications may cause the assembly of tau in human brain.

Besides phosphorylation, other modifications may also be involved. Early studies on tau acetylation reported that it can promote both phosphorylation and aggregation (Min et al. 2010; Cohen et al. 2011). However, subsequent work suggested an inverse correlation between tau acetylation and phosphorylation, with acetylation inhibiting tau assembly (Cook et al. 2014; Carlomagno et al. 2017). These discrepancies may have resulted from the use of enzymes that acetylated different residues. The site-specific acetylation of K280 has been shown to enhance tau aggregation while reducing microtubule assembly (Haj-Yahya and Lashuel 2018). Unlike phosphorylation, acetylation occurs on lysine residues.

In AD, CTE, tangle-only dementia, and many other tauopathies, all six tau isoforms are present in disease filaments (Table 1). The Pick bodies of PiD are only made of 3R tau. In PSP, CBD, AGD, and several other diseases, isoforms with 4R tau are found in the filaments. The morphologies of tau filaments vary in the different diseases, even when they are made of the same isoforms.

GENETICS OF *MAPT*

The relevance of tau inclusion formation for neurodegeneration became clear in June 1998, when dominantly inherited mutations in *MAPT* were shown to cause a form

Table 1. Neurodegenerative diseases with abundant Tau inclusions

3R + 4R tauopathies
Alzheimer's disease
Amyotrophic lateral sclerosis/parkinsonism–dementia complex (Guam and Kii peninsula)
Anti-IgLON5-related tauopathy
Chronic traumatic encephalopathy
Diffuse neurofibrillary tangles with calcification
Down's syndrome
Familial British dementia
Familial Danish dementia
Gerstmann–Sträussler–Scheinker disease
Niemann–Pick disease, type C
Nodding syndrome
Non-Guamanian motor neuron disease with neurofibrillary tangles
Postencephalitic parkinsonism
Primary age-related tauopathy
Progressive ataxia and palatal tremor
Tangle-only dementia
Familial frontotemporal dementia and parkinsonism (some <i>MAPT</i> mutations, such as V337M and R406W)
3R tauopathies
Pick's disease
Familial frontotemporal dementia and parkinsonism (some <i>MAPT</i> mutations, such as G272V and Q336R)
4R tauopathies
Age-related tau astrogliopathy
Argyrophilic grain disease
Corticobasal degeneration
Guadeloupean parkinsonism
Globular glial tauopathy
Hippocampal tauopathy
Huntington's disease
Progressive supranuclear palsy
SLC9a-related parkinsonism
Familial frontotemporal dementia and parkinsonism (some <i>MAPT</i> mutations, such as P301L and P301S, all known intronic mutations, and many coding region mutations in exon 10)

of frontotemporal dementia that can be associated with parkinsonism (FTDP-17T, also known as familial FTLT-tau) (Hutton et al. 1998; Poorkaj et al. 1998; Spillantini et al. 1998). In FTDP-17T, abundant filamentous tau inclusions are present in either nerve cells or in both nerve cells and glial cells. A β deposits, a defining feature of AD, are not characteristic of FTDP-17T. This work established that a pathological pathway leading from monomeric to assembled tau is sufficient for causing neurodegeneration and dementia.

Sixty mutations in *MAPT* have been identified in FTDP-17T (Fig. 1B). Filaments are composed of either 3R or 4R tau or of both 3R and 4R tau. *MAPT* mutations account for ~5% of cases of FTLT and are concentrated in exons 9–12 (encoding R1–R4) and the introns flanking exon 10. They can be divided into those with a primary effect at the protein level and those affecting the alternative splicing of tau pre-mRNA. There is no obvious correlation between known mutations and posttranslational modifications of tau.

The architecture of *MAPT* on chromosome 17q21.31 is characterized by two haplotypes as the result of a 900-kb inversion (H1) or noninversion (H2) polymorphism (Stefansson et al. 2005). Inheritance of the H1 haplotype of

MAPT is a risk factor for PSP, CBD, PD, and amyotrophic lateral sclerosis (ALS), but not for PiD (Conrad et al. 1997; Baker et al. 1999; Pastor et al. 2000; Houlden et al. 2001; Morris et al. 2002; Zhang et al. 2017a). The H2 haplotype is associated with increased expression of exon 3 of *MAPT* in gray matter, suggesting that inclusion of exon 3 may protect against PSP, CBD, PD, and ALS (Caffrey et al. 2008). In experimental studies, exon 3-containing tau isoforms (those with both amino-terminal inserts) have been found to aggregate less than those lacking this exon (Zhong et al. 2012).

Heterozygous microdeletions of chromosome 17q21.31 give rise to a multisystem disorder with intellectual disability, hypotonia, and distinct facial features (17q21.31 microdeletion syndrome or Koolen-de Vries syndrome) (Koolen et al. 2006; Sharp et al. 2006; Shaw-Smith et al. 2006). In addition to *MAPT*, three protein-coding genes (*CRHR1*, *SPPL2C*, and *KANSL1*) and two putative genes (*MGC57346* and *CRHR1-IT1*) are found in this region. Deletions arise on the H2 haplotype through low-copy, repeat-mediated, nonallelic homologous recombination. The 17q21.31 microdeletion syndrome is caused by haploinsufficiency of *KANSL1*, which encodes a chromatin modifier that influences gene expression through the acetylation of lysine 16 of histone H4 (Koolen et al. 2012; Zollino et al. 2012). A 50% reduction in tau levels does therefore not appear to have a detrimental effect on development of the human brain.

Disease-causing mutations in *MAPT* have made it possible to produce transgenic rodent lines that form tau filaments and show neurodegeneration (Goedert et al. 2017). Aggregation of tau correlates with neurodegeneration. Reducing aggregation and increasing degradation of aggregates are therefore therapeutic objectives. It has been reported that the removal of senescent brain cells leads to a reduction in both tau aggregates and neurodegeneration in transgenic mice (Bussian et al. 2018).

Transgenic mouse lines were also essential for the *in vivo* discovery of the prion-like properties of assembled tau. Aggregation of hyperphosphorylated tau was induced following intracerebral injection of tau seeds from mice transgenic for human mutant 0N4R P301S tau into transgenic mice expressing wild-type nonaggregated 2N4R tau and, to a lesser extent, following intracerebral injection into wild-type mice (Clavaguera et al. 2009). Tauopathy then spread to connected brain regions, indicative of seed endocytosis, seeded aggregation, intracellular transport, and release of tau seeds. This work was complemented by studies in cells, which showed that short tau filaments had the greatest seeding activity (Jackson et al. 2016). These findings may be mechanistically related to the observation that in the process leading to AD, tau inclusions first appear in locus coeruleus and entorhinal cortex, followed by the hippocampal formation and large parts of the neocortex (Braak and Braak 1991).

Distinct conformers of assembled tau appear to exist, reminiscent of prion strains. They may explain the variety of human tauopathies. Inclusions formed and spread of pathology occurred after intracerebral injection of brain

homogenates from cases of AD, tangle-only dementia, PSP, CBD, and AGD into a mouse line transgenic for wild-type 4R tau and, to a lesser extent, following intracerebral injection into nontransgenic mice (Clavaguera et al. 2013). PiD, the filamentous inclusions of which consist of only 3R tau, was an exception. Inclusions formed at the injection sites, but spreading was not observed. However, PiD is rarely a pure 3R tauopathy, because it is often associated with a low level of AD-type pathology. We therefore cannot exclude that the aggregation-inducing activity in the PiD homogenate may have been due to the presence of a small amount of aggregated 4R tau. The sequence requirements for seeded tau aggregation *in vivo* remain to be defined. Tau assemblies reminiscent of those in the corresponding human diseases were observed following the injection of brain homogenates from patients with PSP, CBD, and AGD, which are 4R tauopathies. Although these findings are consistent with the existence of distinct tau aggregate conformers, the definition of an aggregate conformer ought to be structural.

HIGH-RESOLUTION STRUCTURES OF TAU FILAMENTS FROM ALZHEIMER'S DISEASE

By cryo-EM, high-resolution structures of tau filaments were obtained from the frontal cortex of four individuals with AD, three sporadic and one inherited (mutation V717F in *APP*) (Fig. 2; Fitzpatrick et al. 2017; Falcon et al. 2018a). The cores of tau filaments are made of two protofilaments consisting of residues G273/304–E380, which adopt a combined cross- β / β -helix structure (Fig. 3). Mouse tau is identical in sequence to human tau in this region. The amino-terminal part of the cross- β structure includes the hexapeptide ³⁰⁶VQIVYK³¹¹ (PHF6), which is essential for the oligomerization of recombinant tau and its assembly into filaments (Fig. 3; von Bergen et al. 2000). It packs against ³⁷³THKLTF³⁷⁸, in agreement with the predicted heterozygote interaction between ³⁰⁶VQIVYK³¹¹ and ³⁷⁵KLTFR³⁷⁹ (Moore et al. 2011). Constructs K18 and K19 end at E372 (Gustke et al. 1994); they can therefore not give rise to the human brain tau folds determined thus far.

Each protofilament contains eight β -strands, five of which give rise to two regions of antiparallel β -sheets, with the other three forming a β -helix (Fig. 4). The carboxy-terminal residues of R1 and R2 form part of the first β -strand. R3 contributes three and R4 four β -strands, with the final β -strand being formed by the 12 amino acids after R4; β 1 and β 2 pack against β 8, β 3 packs against β 7, with β 4, β 5, and β 6 giving rise to the C-shaped β -helix.

Paired helical filaments (PHFs) and straight filaments (SFs) are made of identical protofilaments, but differ in interprotofilament packing, showing that they are ultrastructural polymorphs. PHF protofilaments are arranged base-to-base and SF protofilaments back-to-base. In PHFs, protofilaments are stabilized by backbone hydrogen bonds between their ³³²PGGGQ³³⁶ sequences. Moreover, the side chains of K331 from one protofilament project toward the side chains of Q336 and E338 of the

Figure 2. Cryo-EM structures of paired helical filaments (PHFs) and straight filaments (SFs) from the frontal cortex of four Alzheimer's disease (AD) cases. All structures show identical pairs of C-shaped protofilaments and the same interprotofilament packing in PHFs and SFs. Cases 1, 2, and 3 had sporadic AD, whereas case 4 had inherited AD (mutation V717F in the amyloid precursor protein gene). All cases had a majority of PHFs and a minority of SFs. Yellow arrows point to the extra densities, which were present in PHFs and SFs from all four cases, bordering the solvent-exposed side chains of R349 and K375 and of H362 and K369.

other protofilament, suggesting additional interactions that stabilize the protofilament interface. Furthermore, in the protofilament interface of the PHF, extra densities between the side chains of K331 of one protofilament and the backbone of V337 of the other have been observed. They may correspond to a solvent molecule or a posttranslational modification of K331, such as monomethylation (Falcon et al. 2018a).

In SFs, the protofilaments pack asymmetrically. Their backbones are nearest each other between residues ³²¹KCGS³²⁴ of the first and ³¹³VDLSK³¹⁷ of the second protofilament. The interprotofilament packing appears to be stabilized through the region of additional density that interacts with the side chains of K317, T319, and K321 of both protofilaments. This density may correspond to resi-

dues ⁷EFE⁹, which constitute the amino-terminal region of the discontinuous epitope of the conformational anti-tau antibodies ALZ-50 and MC-1 (the carboxy-terminal epitope is ³¹³VDLSKVTSKC³²²) (Jicha et al. 1997). A similar density also interacts with K317, T319, and K321 in PHFs, where it does not contribute to the protofilament interface.

HIGH-RESOLUTION STRUCTURES OF TAU FILAMENTS FROM PICK'S DISEASE

By cryo-EM, high-resolution structures of tau filaments were determined from the frontotemporal cortex of an individual with PiD (Falcon et al. 2018b). Two types of filaments could be distinguished: a majority of narrow

Figure 3. Cryo-EM densities and atomic models of paired helical filaments (PHFs) and straight filaments (SFs) from the frontal cortex of an individual with Alzheimer's disease. PHFs and SFs were resolved to 3.2 and 3.3 Å, respectively. Sharpened, high-resolution maps are shown in blue (PHFs) and green (SFs). Unsharpened 4.5 Å low-pass filtered densities are shown in gray. The models comprise G273–E380 of 3R tau and G304–E380 of 4R tau. The protofilaments of PHFs and SFs are identical but differ in interprotofilament packing (ultrastructural polymorphs).

Figure 4. Schematic view of the tau protofilament core of Alzheimer's disease. The observed eight β -strand regions (β 1– β 8) are shown as arrows. Each filament consists of two protofilaments.

Pick filaments (NPFs) and a minority of wide Pick filaments (WPFs) (Fig. 5). The core of NPFs is made of a single protofilament that consists of residues K254–F378 of 3R tau, which adopt an elongated cross- β structure. Mouse tau is identical to human tau in this region, with the exception of residue 257 (K in human, R in mouse tau). WPFs are formed by the association of two NPF protofilaments at their distal tips, where they form tight contacts through van der Waals interactions. Each protofilament comprises nine β -strands, which are arranged into four cross- β packing stacks and are connected by turns and arcs (Fig. 6). R1 provides two β -strands and

R3 and R4 three β -strands each. These stacks pack together in a hairpin-like fashion: β 1 against β 8, β 2 against β 7, β 3 against β 6, and β 4 against β 5. The final strand, β 9, is formed from the 10 amino acids after R4 and packs against the opposite side of β 8.

Three regions of less well-resolved density bordering the solvent-exposed faces of β 4, β 5, and β 9 are apparent in both NPFs and WPFs. They may represent less ordered, heterogeneous, and/or transiently occupied structures. The density bordering β 4 is similarly located, but more extended, than that found to interact with the side chains of K317, T319, and K321 in AD filaments.

Figure 5. Narrow (93%) and wide (7%) tau filaments (NPFs and WPFs) are characteristic of Pick's disease. Unsharpened cryo-EM densities of a NPF (A) and a WPF (B). NPFs were resolved to 3.2 Å. (C) Sharpened, high-resolution cryo-EM map of the NPF with the atomic model of the Pick fold overlaid.

Figure 6. Schematic view of the tau protofilament core of Pick's disease. The observed nine β -strands (β 1– β 9) are shown as arrows. Narrow Pick filaments are made of one protofilament. Wide Pick filaments comprise two narrow filaments that are joined at their distal tips.

It was not previously known why only 3R tau is present in Pick body filaments and why S262 is not phosphorylated. Our results suggest that despite sequence homology, the structure formed by K254–K274 of R1 is inaccessible to the corresponding residues from R2 (S285–S305). Moreover, because of steric constraints, the filament structure precludes phosphorylation of S262.

CONCLUSION

Cryo-EM of filaments from human brain has established that distinct conformers of aggregated tau are characteristic of AD and PiD. Even though both types of filaments share residues G273–F378 of 3R tau, their structures are very different (Fig. 7). Whereas PHFs and SFs of AD are made of two identical C-shaped protofilaments

Figure 7. Comparison of Alzheimer and Pick tau filament folds. (A) Microtubule-binding repeats (R1–R4, residues 244–368) and some flanking sequences, with the observed eight β -strand regions (arrows) in the Alzheimer fold and nine β -strand regions (arrows) in the Pick fold, as well as corresponding loop regions, colored from violet to red. (B) Schematic representation of the different tau folds. Paired helical (PHF) and straight (SF) tau filaments of Alzheimer's disease consist of two identical protofilaments that differ in interprotofilament packing (ultrastructural polymorphs). More than 90% of tau filaments of Pick's disease are narrow Pick filaments (NPFs) that consist of a single protofilament. Wide Pick filaments (WPFs) consist of two narrow filaments packed against each other. (C) Schematic of the Alzheimer and Pick folds, depicted as a single rung. C322 (yellow "C") and D348 (red "D") are highlighted.

that each comprises eight β -strands and a combined cross- β -helix structure, NPFs of PiD are made of a single elongated protofilament comprising nine β -strands and stacks of β -sheets. WPFs consist of two NPFs joined through their distal tips. Cryo-EM studies of tau filaments from additional sporadic and inherited cases of AD, as well as negative-stain immuno-electron microscopy of tau filaments from multiple cases of AD and PiD, indicated that the cores of tau filaments from each disease case contain the same sequences. It therefore seems that the structures of tau filaments are distinct between diseases but identical in different individuals with AD or PiD. It appears likely that additional folds of assembled tau remain to be discovered in other tauopathies.

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