REVIEW

Amyloidogenesis of Tau protein

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Abstract: The role of microtubule-associated protein Tau in neurodegeneration has been extensively investigated since the discovery of Tau amyloid aggregates in the brains of patients with Alzheimer's disease (AD). The process of formation of amyloid fibrils is known as amyloidogenesis and attracts much attention as a potential target in the prevention and treatment of neurodegenerative conditions linked to protein aggregation. Cerebral deposition of amyloid aggregates of Tau is observed not only in AD but also in numerous other tauopathies and prion diseases. Amyloidogenesis of intrinsically unstructured monomers of Tau can be triggered by mutations in the Tau gene, post-translational modifications, or interactions with polyanionic molecules and aggregation-prone proteins/peptides. The self-assembly of amyloid fibrils of Tau shares a number of characteristic features with amyloidogenesis of other proteins involved in neurodegenerative diseases. For example, *in vitro* experiments have demonstrated that the nucleation phase, which is the rate-limiting stage of Tau amyloidogenesis, is shortened in the presence of fragmented preformed Tau fibrils acting as aggregation templates ("seeds"). Accordingly, Tau aggregates released by tauopathy-

Abbreviations: A β , amyloid β ; AD, Alzheimer's disease; AEP, asparagine endopeptidase; AFM, atomic force microscopy; AGD, argyrophilic grain disease; ApoE, apolipoprotein E; APP, amyloid precursor protein; BSE, bovine spongiform encephalopathy; CBD, corticobasal degeneration; Cdk5, cyclin-dependent kinase 5; CJD, Creutzfeldt-Jakob disease; FAD, familial form of Alzheimer's disease; FV-AFM, AFM in the force-volume mode; FTDP-17, frontotemporal dementia and parkinsonism linked to chromosome 17; GSK3 β , glycogen synthase kinase 3 β ; GSS, Gerstmann-Straussler-Scheinker disease; HD, Huntington's disease; IDP, intrinsically disordered protein; K18, Tau peptide comprising 4 repeats of the microtubule-binding domain; K19, Tau peptide comprising 3 repeats of the microtubule-binding domain; LTP, long term potentiation; MAPs, microtubule-associated proteins; *MAPT*, Tau gene; MARK, microtubule-associated regulatory kinase; MBD, microtubule-binding domain; MMP-9, matrix-metalloproteinase 9; MTBRs, microtubule-binding repeats; MTs, microtubules; NFTs, neurofibrillary tangles; NMR, nuclear magnetic resonance; PHFs, paired helical filaments; PHF6, a hexapeptide motif comprising a sequence VQIVYK; PHF6*, a hexapeptide motif comprising a sequence VQIINK; PHF43, 43-residue peptide corresponding to K19 (265-338); PiD, Pick's disease; Pin1, peptidylprolyl cis/trans isomerase NIMA-interacting 1; PKA, protein kinase A; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PP2B, protein phosphatase 2B; PP5, protein phosphatase 5; PQC, protein quality control; PrP^C, cellular prion protein; PrP^{Sc}, scrapie isoform of prion protein; *PS1*, presenilin 1 gene; *PS2*, presenilin 2 gene; PSP, progressive supranuclear palsy; PTMs, post-translational modifications; SAD, sporadic form of Alzheimer's disease; SAPK/JNK, stress activated kinase/c-jun N-terminal kinase; SFs, straight filaments; TEM, transmission electron microscopy; TGs, transglutaminases; TSEs, transmissible spongiform encephalopathies; UPS, ubiquitin-proteasome system.

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affected neurons can spread the neurodegenerative process in the brain through a prion-like mechanism, originally described for the pathogenic form of prion protein. Moreover, Tau has been shown to form amyloid strains—structurally diverse self-propagating aggregates of potentially various pathological effects, resembling in this respect prion strains. Here, we review the current literature on Tau aggregation and discuss mechanisms of propagation of Tau amyloid in the light of the prion-like paradigm.

Keywords: Tau protein; amyloidogenesis; protein aggregation; tauopathies; Alzheimer's disease

Introduction

Tau protein, which belongs to the family of microtubule-associated proteins (MAPs), was discovered in 1975 and identified as a molecule necessary for the assembly and stabilization of microtubular cytoskeleton.¹ Subsequently, in Alzheimer's disease (AD), a pathological form of Tau was demonstrated to be the principal component of paired helical filaments (PHFs) which further associate into neurofibrillary tangles (NFTs).^{2,3} These structures constitute one of the main neuropathological hallmarks of AD.⁴ Besides NFTs formation of senile plaques composed of amyloid β (A β) peptides is widely recognized as a characteristic trait of AD pathogenesis.⁵ Deposits of Tau aggregates are also detected in numerous other tauopathies such as frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD), argyrophilic grain disease (AGD), and Down's syndrome (DS).^{6,7} Since abundance of tangles in AD brains clearly correlates with the progression of neurodegenerative changes and memory loss, for many years NFTs have been considered toxic.⁸ However, more recent findings have changed this view, suggesting that certain oligomeric but nonfibrillar and structurally heterogeneous assemblies that appear early and usually transiently during aggregation are the most neurotoxic forms of Tau.^{9–11} Amyloid aggregates of Tau, such as PHFs, are formed in a process known as amyloidogenesis or fibrillization. In the course of this process, intrinsically disordered Tau molecules self-assemble through various intermediates to form highly ordered β -sheet-rich amyloid fibrils.^{12,13} Typically, PHF aggregates are composed of two fibrils wounding around each other. Tau amyloid assemblies can also adopt several other morphologies such as straight filaments (SFs) and ribbon-like fibrils.¹⁴ The ability of Tau to form polymorphic fibrils parallels similar observations made for other amyloidogenic proteins.^{15,16} Amyloid aggregates can propagate their structural features leading to template-dependent conversion of nonphysiological structures.^{17,18} This principle underlies propagation scenarios of mammalian prions which cause transmissible spongiform encephalopathies (TSEs) also known as prion diseases.¹⁹ These include inherited, sporadic and acquired diseases, such as kuru,

Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker disease (GSS), scrapie, and bovine spongiform encephalopathy (BSE).¹⁹ Pathobiology of prions is linked to the phenomenon of prion strains: transmissible conformational variants of prion protein distinct in terms of pathological patterns. The existence of prion strains has been proposed to explain the observed phenotypic diversity in TSEs.^{20,21} Besides prion protein, other amyloidogenic proteins are able to form polymorphic amyloid aggregates capable of self-propagation to daughter generations of fibrils. It is believed that the underlying molecular mechanisms resemble those implicated in prion replication. The ability to form amyloid strains has been demonstrated for numerous proteins.^{22–25} In this regard, it has been proposed that they can be collectively called prion-like proteins.^{26,27} Given the fact that Tau can aggregate via template-based conversion to form amyloid polymorphs, spreading within specific regions of the brain, it has been proposed that Tau represents an example of the prion-like protein.^{22,28,29} Nevertheless, the mechanisms of Tau amyloidogenesis, as well as molecular factors which can trigger conformational conversion of unstructured Tau monomers leading to the formation of amyloid assemblies, remain elusive.

Domain Structure of Tau

The microtubule-associated protein Tau gene (*MAPT*) is located on chromosome 17q21.³⁰ Alternative splicing of *MAPT* leads to the generation of six major isoforms of Tau in the adult human brain.^{31,32} Splicing of exons 2 and 3, which code for N-terminal inserts, results in Tau isoforms with two, one or none N-terminal inserts (2N, 1N, 0N).³³ Alternative splicing of exon 10 gives isoforms with four (exon 10 inclusion, 4R) or three microtubule binding repeats (MTBRs) (exon 10 exclusion, 3R).³⁴ The six isoforms range from 352 to 441 amino acid residues in length.³⁵ Each Tau isoform contains a projection domain (encompassing above-mentioned acidic insertions denoted as N), proline-rich regions and a microtubule binding domain (MBD) composed of either three (3R) or four (4R) repeats of 31 or 32 residues located in the C-terminal part of the molecule (Fig. 1).^{36–39} The MBD and the proline-rich regions are both positively charged. The N-terminal part

Figure 1. The domain organization of Tau. **(A)** There are 6 major Tau isoforms in the human brain: 2N4R, 1N4R, 0N4R, 2N3R, 1N3R, 0N3R. Molecules of these isoforms consist of two main domains: the projection domain that protrudes from the microtubule surface and the microtubule-binding domain (MBD) which has high affinity for MTs. The isoforms differ in the number of N-terminal inserts (N) and repeats (R) within microtubule-binding repeats (MTBRs) region. The MTBRs are located within the MBD. The 4 repeats (4R) isoforms contain two hexapeptide motifs (PHF6* and PHF6) and two cysteines (C291 and C322), whereas 3R isoforms have only one hexapeptide motif (PHF6) and cysteine (C322). The longest isoform (2N4R, 441 amino acid residues) contains two inserts (2N) at the N terminus which are followed by the proline-rich region (P1, P2) and MTBRs (composed of 4R). The third proline-rich region (P3) follows the R4. Regions rich in negative (-) and positive (+) charges are indicated. Tau peptides and polypeptides used as models to study amyloidogenesis include PHF6* and PHF6 **(A)** as well as K18 (4R), K19 (3R) and PHF43 **(B)**. The predicted structure of Tau polypeptide (267-312, corresponding to a fragment of the repeat region) in a MT-bound conformation revealed by NMR spectroscopy is shown in **(C)** (PDB: 2MZ7,²⁹²). Note local enrichment in secondary structures. The structure was visualized in PyMOL²⁹³

and a short region at the C-terminus are acidic. A series of recombinant peptides encompassing the repeat region was constructed including K18 and K19. It turned out that K18 and K19 are prone to aggregation since they do not contain the flanking regions that inhibit amyloidogenesis and they correspond to an amyloid core of PHFs.³⁶ Studies on aggregation of such polypeptides provide important insights into the amyloidogenesis of Tau.

It has been demonstrated that the C-terminal fragment of Tau comprising residues 422-441 adopts α -helical conformation and inhibits Tau amyloidogenesis (isoform 2N4R) through interaction with residues 321-375 encompassing the MTBR region.⁴⁰ In addition, the N-terminal fragment of Tau, containing residues 1-196, inhibits Tau fibrillization by

interaction with residues 392-421 at the C-terminal part, which may lead to the stabilization of Tau monomers.⁴¹ Consequently, it has been proposed that association of the C-terminal part of Tau with the MTBRs prevents the aggregation. Once C-terminal region moves away from the MTBRs, the N-terminus may associate with the repeat region, which in turn may allow Tau to adopt an amyloid-prone conformation.⁴¹

Brain-derived Tau and recombinant Tau polypeptides do not adopt well-defined structures under physiological conditions.³⁶ The intrinsically disordered character of Tau primarily arises from the excess of positively charged residues and relatively small fraction of bulky hydrophobic amino acid side-chains.^{42,43} In addition, Tau is rich in proline

residues which locally prohibit formation of α -helix or β -sheet. As a consequence of its amino acid composition, Tau monomers are dynamically interchanging disordered conformations with large solvent-exposed surfaces.^{44–46} The fragment of Tau molecule encompassing the microtubule binding repeats adopts extended conformation as revealed by solution small-angle X-ray scattering. Interestingly, the size of the full-length molecule of Tau is smaller than could be expected, which may be explained by a paperclip/global hairpin structure whose N- and C-termini bend inward in the direction of the repeat region, compensating for its extended conformation.⁴⁷ Conversely, it has been demonstrated that Tau can adopt a transiently ordered structure upon binding to microtubules which forces the unstructured polypeptide chain of Tau to adopt a biologically functional state with low content of defined secondary structures.^{48–50}

Tau as an intrinsically disordered protein (IDP) is “sufficiently disordered” for initial stages of aggregation and therefore its aggregation does not face energy barriers related to the partial unfolding which usually precedes amyloidogenesis of structured proteins. In fact, it has been proposed that Tau fibrillization must be associated with folding events which would allow monomers of Tau to acquire partially folded amyloid-prone structure.⁵¹ Furthermore, the large Coulombic charges on each Tau monomer may cause significant obstacles due to electrostatic repulsion which must be overcome or reduced prior to association of the molecules into aggregates.

Molecular Bases of Tau Pathology in Tauopathies

Neurodegenerative diseases linked to protein aggregation are frequently characterized by impairments of the protein quality control (PQC) mechanisms.⁵² However, it is not clear whether alterations in PQC are primary or secondary events of neurodegeneration. *In vitro*-generated or AD-derived amyloid fibrils of Tau, as well as Tau oligomers can inhibit the ubiquitin–proteasome system (UPS).^{53,54} It is also known that distinct forms of Tau: native, carrying mutations and/or aggregated can be degraded by autophagy, which may directly contribute to the reduction of Tau-linked toxicity.^{55,56} Consistently, neurons are sensitive to autophagy impairments which may occur in neurodegenerative diseases such as AD and other tauopathies.⁵⁷ Molecular chaperones may also prevent amyloidogenesis and loss of their function is frequently observed during neurodegenerative disorders.⁵⁸ Chaperones have been shown to decrease Tau hyperphosphorylation and aggregation levels, increase the concentration of soluble Tau and support binding of Tau to MTs.^{59–61}

As mentioned above, tauopathy-associated aggregates of Tau are structurally-heterogeneous. Tau can assemble into PHFs, SFs or other linear aggregates.¹⁴ In addition, lesion morphology may be diverse, including AD-typical tangles (NFTs), spindle-shaped, irregular or diffused deposits.⁶² Another aspect of the structural heterogeneity of these aggregates manifests on the level of cellular pathology. Pathological changes may affect exclusively neurons, or both neurons and glial cells.⁶² Furthermore, distinct brain regions are affected by pathological aggregates of Tau.⁶³ Although many factors have been shown to influence Tau amyloidogenesis, it is unclear which of them are primary triggers that impact the progression of tauopathies and precise mechanisms that explain variability among tauopathies remain enigmatic.

According to the amyloid cascade hypothesis,⁵ increased production and aggregation of A β lead to the accumulation of toxic species of this peptide which further causes brain pathology and dementia. This hypothesis is based on the assumption that Tau pathology in AD is a result of A β aggregation. The principal role of A β in AD is particularly clear in the case of familial form of Alzheimer’s disease (FAD). In this form of AD, autosomal dominant mutations in one of three genes: *PS1* (presenilin 1), *PS2* (presenilin 2), or *APP* (amyloid precursor protein) are sufficient to cause symptoms of the disease.⁵ The potential risk factors for sporadic Alzheimer’s disease (SAD) include $\epsilon 4$ allele of apolipoprotein E (ApoE) which plausibly can influence A β clearance mechanisms.⁶⁴ Importantly, some changes in *MAPT* expression or alternative splicing have also been suggested to be associated with the risk for AD.⁶⁵ However, currently there is no described mutation in *MAPT* involved in AD. It is unclear whether, or to what extent, Tau pathology in AD might be independent of A β aggregation. However, it is accepted that AD pathology is inseparably linked to neuronal death mediated by Tau oligomerization/aggregation. Clearly, Tau is essential for toxic effects of A β ⁶⁶ since Tau-depleted hippocampal neurons are not prone to neurodegeneration upon treatment with A β amyloid aggregates.⁶⁷ There seems to be an interplay between Tau and A β aggregation—both *in vitro* and *in vivo*. For example, A β pathology may facilitate Tau pathology in a mouse model of AD by accelerating Tau fibrillization and leading to reduction of density of dendritic spines and cognitive impairment.⁶⁸ According to some studies, A β oligomers can stimulate Tau hyperphosphorylation in hippocampal neurons.⁶⁹ Furthermore, A β dimers have been shown to induce hyperphosphorylation of Tau, impair the MT network, leading to neuritic degeneration in hippocampal neurons.⁷⁰ Prevention of cognitive impairments in transgenic mice with familial AD mutations and overexpressing hAPP was

achieved through the reduction of Tau expression, even at high production of A β .⁷¹ These findings support the importance of Tau amyloidogenesis in AD and suggest that mutual relationship between Tau and A β aggregation may underlie neuropathology in this disease.

In contrast to AD, numerous pathogenic mutations in *MAPT* gene have been found in the autosomal dominantly inherited dementia FTDP-17, proving that Tau pathology alone is sufficient to cause neurodegeneration.⁶³ There are over 50 identified mutations in *MAPT* that are responsible for FTDP-17, and most of them occur in the microtubule binding domain of Tau.^{63,72} It has been demonstrated that mutations in *MAPT* can stimulate fibrillization of Tau even in the absence of polyanionic inducers.⁷³ Some Tau mutants have increased tendencies to aggregate into PHFs.⁷⁴ In FTDP-17, Tau pathology is found in both neurons and glia, varying significantly even among patients with the same mutation in *MAPT*, which suggests that additional factors may contribute to manifestation of this disorder.^{7,75} Interestingly, it has been reported that in FTDP-17, deposits of A β amyloid plaques can also be detected within the brain besides typical aggregates composed of Tau.⁷⁶ This may imply that amyloid aggregates of Tau can induce A β -related pathology. FTDP-17 mutations may lead to distinct effects on Tau fibrillization.⁷⁷ Mutated Tau is

hyperphosphorylated in brains of FTDP-17 patients⁷⁸ and exhibits a diminished ability to promote assembly of MTs,⁷⁹ while forming polymorphic amyloid fibrils resembling those found in AD.⁷³ Some mutations in *MAPT* may lead to aberrant splicing of Tau pre-mRNA, resulting in the increased ratio of 4R to 3R Tau isoforms, which is approximately 1:1 in the normal adult brain.^{78,80,81}

A Model of Tau Amyloidogenesis

According to a widely accepted model, Tau amyloidogenesis proceeds via a nucleated polymerization mechanism which is common for many amyloidogenic proteins. The process of Tau fibrillization displays sigmoidal kinetics and is characterized by three major stages: a lag phase, an elongation phase, and a plateau phase.^{82,83} In a process called seeding, upon adding mature amyloid fibrils (seeds) to precursor protein molecules, the elimination of the lag phase and rapid initiation of amyloid growth are observed.⁸⁴ The recruitment of soluble protein molecules leads to the growth of seeds, which may undergo fragmentation to generate more seeds (Fig. 2).

Detection of transient entities which appear during the lag phase is difficult and molecular events taking place during this stage remain poorly characterized. As stated earlier, disease-associated rearrangements within the Tau molecule may lead to association of its amino terminus with MTBRs.⁴¹

Figure 2. The scheme of nucleated formation of Tau amyloid aggregates. **(A)** Intrinsically unstructured monomers of Tau (indicated in blue) do not acquire one specific conformation, but rather undergo transitions from one accessible conformational state to another. It is assumed that the formation of structured amyloid assemblies from unstructured monomers of Tau requires partial folding. It has been proposed that an amyloid-competent conformer of Tau (indicated in green) is initially formed. It is also hypothesized that such competent monomers assemble into nuclei of Tau amyloidogenesis. Formation of the nuclei is a rate-limiting stage and is followed by the rapid growth of fibrils. The tips of the amyloid fibril can act as templates incorporating the competent conformers leading to the elongation of the fibril. Hence, the number of available amyloid ends determines the rate of amyloidogenesis. Fragmentation of amyloid aggregates (usually accomplished *in vitro* by sonication of mature fibrils) produces short fragments called seeds, which can recruit native and amyloidogenic monomers leading to rapid growth of amyloid assemblies. **(B)** The scheme of kinetics of unseeded and seeded fibrillization of Tau

This conformation has been suggested to promote ordered aggregation of Tau in the presence of inducing agents.⁴¹ Formation of amyloid nuclei during the lag phase is slow (due to barriers of mainly entropic nature), but once they are formed, the rapid assembly of amyloid fibrils follows.^{15,85,86} Therefore, formation of nuclei is the principal rate-limiting step in amyloidogenesis. It has been reported that dimerization of Tau molecules, through intermolecular disulfide bonds, leads to a local antiparallel alignment. This dimerization has been proposed to precede formation of the nucleus.^{87,88} Subsequently, it has been speculated that this nucleus consists of 4 to 7 dimers.⁸⁹ Both Tau dimers with inter- and intramolecular disulfide bonds have been proposed to retain ability to form fibrils.⁹⁰ Conversely, the formation of an intramolecular disulfide bridge between two cysteines in 4R Tau isoforms may lead to the generation of “compact monomers” which were proposed to be fibrillization-incompetent.⁹¹ It should be stressed, however, that the role of dimerization in Tau amyloidogenesis was called into question, regarding very low probability of intermolecular disulfide bonds formation via two cysteines located in the highly positively charged repeat region of 4R Tau molecules.⁹² Presently, it is clear that formation of disulfide bridges is not crucial for Tau amyloidogenesis to occur. Instead, it has been found that certain short amino acid sequences are particularly important for the initiation of early stages of Tau aggregation. Regions known as hexapeptide motifs, PHF6 (VQIVYK) and PHF6* (VQIINK), may act as nucleating segments for Tau assembly. These sequences are located within the microtubule binding domain (Fig. 1). PHF6 and PHF6* have been proposed to promote aggregation by inducing nucleation events as they have increased tendency to form conformations rich in β -sheets.^{73,93,94} Soluble protein molecules of Tau are progressively converted into amyloid fibrils during the elongation phase. It has been proposed that the growth of Tau fibrils proceeds through a zipper-like mechanism in which unfolded molecules of Tau form parallel in-register β -strands.⁹⁵ Successive incorporation of Tau monomers to the tips of fibrils via this mechanism enables growth of the fibril. Finally, amyloidogenesis stops at the plateau phase when maximal amount of protein monomers is assembled into amyloid aggregates.⁸²

Each of the stages of Tau aggregation may be modulated by several factors, including: concentration of precursor protein molecules,⁹⁶ temperature,⁹⁷ pH,⁹⁸ salts,⁹⁸ shear force,⁹⁹ as well as additional substances that may either stimulate or inhibit aggregation.^{100,101} *In vitro* studies on Tau fibrillization under nonphysiological or near-physiological conditions provided important insights into the mechanism of aggregation processes. Conversely, physiologically relevant conditions, under which proteins form

amyloid fibrils in the brain, have remained challenging to investigate.¹⁰² This can be explained by very low rates of amyloidogenic processes in the cellular environment which evolved to protect native proteins from forming cytotoxic species.^{103,104}

The Role of Polyanions in Tau Amyloidogenesis

The high abundance of basic amino acid residues within the primary structure of Tau is the main cause of its intrinsically disordered character. This circumstance is expected to have both aggregation-enhancing (by making Tau monomers more structurally dynamic) and aggregation-preventing (due to the electrostatic repulsion) consequences. Hence, the excessive positive charge on nascent Tau molecules needs to be effectively attenuated either through an extensive phosphorylation (as is the case of Tau found in NFTs), or through interactions with anionic species (proteinaceous or otherwise). Most studies focusing on the latter scenario look into possible role of physiologically-relevant poly- and oligo-anions such as glycosaminoglycans (mostly heparin and heparan),^{105–108} but also smaller anions with a single negative charge which could be present and involved during *in vivo* aggregation of Tau—for example arachidonic acid,¹⁰⁹ docosahexaenoic acid¹¹⁰ or even taurine.¹¹¹ It should be noted, however, that in the case of amphiphilic anionic inducers (this category encompasses fatty acids and synthetic detergents—e.g., Ref. 112) the inducer-Tau association is likely to be preceded by formation of micellar assemblies by the former, i.e., the protein interacts with a macromolecular system with a large net negative charge. There are several studies exploring Tau aggregation under strictly *in vitro* conditions which employ for this purpose synthetic anionic inducers such as poly-L-glutamic acid^{98,113} or Congo Red (the latter reviewed in work¹¹⁴). These studies, even if conducted under somehow artificial conditions, may provide important clues into the structural dynamics of aggregation-prone Tau conformers. In particular, this approach could prove very insightful in regard to certain opaque issues surrounding dynamics of aggregation of Tau which cannot be easily addressed using RNA or heparin, for example the role of chirality and chiral transfer between the inducer and Tau in determining fibrillization pattern. Investigation of mechanisms of Tau aggregation induced by polyglutamic acid may also have important biological implications due to the presence of a polyglutamic sequence located in the C-terminal region of the tubulin molecule that is involved in binding to the repeat region of Tau.^{98,115,116}

However, out of all polyanionic inducers of Tau aggregation, heparin is the most frequently studied and considerably relevant for the physiological conditions, and is therefore the one attracting most attention. Heparin fractions composed of sufficiently

Table I. PTMs of Tau

PTM	Sites	References
Phosphorylation	T17, Y18 , Y29, T30, T39, S46 , T50, T52, S56, S61, T63, S64, S68 , T69 , T71 , T76, T95, T101, T102, T111 , S113 , T123 , S129, S131, T135, S137 , T149, T153, T169, T175 , T181 , S184 , S185 , S191 , S195, Y197 , S198 , S199 , S202 , T205 , S208 , S210 , T212 , S214 , T217 , T220, T231 , S235 , S236 , S237 , S238 , S241, T245, S258 , S262 , T263, S285, S289 , S293, S305, Y310, S316, T319, S320, S324, S341, S352, S356 , T361, T373, T377, T386, Y394 , S396 , S400 , T403 , S404 , S409 , S412 , S413 , T414 , S416 , S422 , T427 , S433 , S435	122–126
Proteolytic cleavage	D13 , N255, D348, N368 , E391 , D402 , D421	127–130
Glycosylation	T181, S199, S202, T205, T212, S214, T217, S262, S356, S404, S422	131,132
Acetylation	K148, K150, K163, K174 , K180, K190, K234, K240, K254, K257, K259, K267, K274 , K280 , K281 , K290, K298, K311, K317, K321, K331, K353, K369, K370, K383, K385, K395	124,133–136
Prolyl-isomerization	T231	137,138
SUMOylation	K340	139
Ubiquitination	K254 , K311 , K353	124,140,141
Methylation	K24, K44 , K67, K163 , K174 , K180 , K190, K254 , K259, K267 , K290 , K311, K317, K353, K385	142,143
Polyamination	Q6, K24, Q88, Q124, K163, K174, K180, K190, K225, K234, K240, Q244, Q276, Q288, Q351, K383, K385, Q424	131,144
Glycation	K87, K132, K150, K163, K174, K225, K234, K259, K280, K281, K347, K353, K369	145
Nitration	Y18 , Y29 , Y197 , Y310, Y394	146,147

Numbering of amino acid residues corresponds to the sequence of the longest human Tau isoform (2N4R, 441 residues). Residues displayed in bold have been found to be modified in AD.

long chains are known to be potent polyanionic inducers of Tau fibrillization, which contrasts with the behavior of shorter forms of this glycosaminoglycan.^{105,117} Apart from providing compensation of electric charges, heparin chains may act as flexible molecular rulers with periodicity matching that of the protein partner.^{105,118} Ramachandran and Udgaonkar¹¹⁹ have shown in their study on four repeat domain of Tau (Tau4RD) that the heparin effect sets in very early in the course of aggregation by promoting binding of two Tau monomers and subsequent formation of an aggregation-prone dimer. Heparin is particularly useful in studies of aggregation of truncated versions of Tau, and, in general, *in vitro* heparin-induced aggregates tend to reveal the proper characteristics of PHFs rather than single filament morphology typically found in arachidonic acid-induced aggregates (see¹¹⁸ for further discussion). A study by Sibille et al.¹⁰⁵ probed with high resolution (afforded by the application of NMR spectroscopy) the precise nature of interactions between full-length Tau and heparin. The authors have identified major regions within Tau responsible for binding the glycosaminoglycan which include highly charged sequences flanking MTBRs. Furthermore, it was shown that initial interactions with heparin are likely to foster formation of both β -strands and α -helices in various locations in Tau which, in the absence of heparin, remain disordered. Finally, the study provided strong evidence that during the aggregation process heparin is buried within the rigid core of growing amyloid fibril.

Further studies are needed to untangle the complex interplay of interactions between Tau and polyanionic molecules during an amyloidogenic pathway and shed light on the possible role of naturally occurring oligoanions in selection of various polymorphs of Tau amyloid.

Post-Translational Modifications of Tau and Their Role in Amyloidogenesis

The physiological functions of Tau are regulated by various post-translational modifications (PTMs) which may also affect its conformational state. On the one hand, PTMs may regulate affinity of Tau to MTs.¹²⁰ On the other hand, an abnormal level of some modifications may trigger Tau aggregation.³⁷ There are contradictory reports on the relevance of some PTMs in Tau amyloidogenesis.¹²¹ PTMs of Tau (Table I) include phosphorylation, proteolytic cleavage, glycosylation, acetylation, prolyl-isomerization, SUMOylation, ubiquitination, methylation, polyamination, glycation, and nitration.

Phosphorylation of Tau

Phosphorylation and dephosphorylation play an important physiological function by modulating affinity of Tau to MTs.¹⁴⁸ Hyperphosphorylated Tau accumulates in the form of NFTs in AD and related tauopathies.^{149–151} Deposits of hyperphosphorylated Tau are also detected in prion diseases.¹⁵² It is widely known that hyperphosphorylated Tau dissociates from MTs which may result in the breakdown of microtubular cytoskeleton.^{153,154} Furthermore,

in vitro studies have demonstrated that hyperphosphorylated Tau can co-aggregate with normal Tau leading to the sequestration of the functional molecules, while a treatment of hyperphosphorylated Tau with alkaline phosphatase prevents formation of such co-aggregates.¹⁴⁹ Abnormally elevated phosphorylation compensates for the positive charges on the Tau molecule and thereby may lead to conformational changes of Tau triggering its aggregation.¹⁵⁵ A similar effect is observed for above-mentioned polyanion-induced amyloidogenesis of Tau.¹⁵⁶ Numerous protein kinases which can phosphorylate Tau have been identified, including glycogen synthase kinase 3 β (GSK3 β),¹⁵⁷ protein kinase A (PKA),¹⁵⁸ cyclin-dependent kinase 5 (cdk5),¹⁵⁹ microtubule-associated regulatory kinase (MARK),¹⁶⁰ and stress activated kinase/c-jun N-terminal kinase (SAPK/JNK).¹⁶¹ Phosphatases which can dephosphorylate Tau in the human brain include PP1, PP2A, PP2B, PP5.¹⁶² Among them PP2A is considered as the crucial one.¹⁶³ It has been proposed that Tau aggregation may be enhanced by the phosphorylation within the C-terminal part of the molecule, including the microtubule-binding region, and suppressed when the N-terminal part and the proline-rich regions are phosphorylated.^{130,164} In tauopathies, Tau becomes hyperphosphorylated at multiple serine and threonine residues.¹⁶⁵ Phosphorylation of KXGS motifs, which are located in the microtubule binding domain, precedes Tau amyloidogenesis and promotes dissociation of Tau from MTs.¹⁶⁶ Tau in AD-derived PHFs can be phosphorylated at ~45 residues.¹²⁵ Most of phosphorylation sites on Tau molecules are found in the proline-rich region and the C-terminal part. Only four phosphorylation sites: S258, S262, S289, S356 are found in the repeat region.¹²⁵ However, phosphorylation at these residues may significantly affect MT-binding abilities of Tau molecules. One of the first sites phosphorylated in AD is a residue S262 (located within the microtubule-binding domain).¹⁶⁷ Phosphorylation of numerous other sites has been suggested to influence AD pathogenesis. It remains unclear whether an elevated level of phosphorylation is absolutely required or sufficient to induce Tau aggregation *in vivo* and to what extent phosphorylated species of Tau are neurotoxic. Furthermore, the phosphorylation at some sites which is thought to cause detachment of Tau from MTs, may also protect Tau from aggregation into PHFs.¹⁶⁸ Interestingly, increased level of Tau phosphorylation occurs also in normal fetal brains in the absence of Tau pathology.¹⁶⁹ In some animals, Tau is hyperphosphorylated during hibernation as a neuroprotective strategy.¹⁷⁰

Proteolytic cleavage of Tau

Proteolytic cleavage can facilitate Tau amyloidogenesis and provide neurotoxic species. The protein is a substrate for several caspases and can be cleaved at

residue D421 (isoform 2N4R) in neurons treated with A β 1-42 aggregates. It has been shown that Tau 1-421 assembles during arachidonic acid-induced fibrillization more readily than the full-length molecule.¹²⁹ Caspases, including caspase-3 and -6, have been linked with the proteolytic cleavage of Tau in AD. Caspase-3 cleaves Tau at residues D25 and D421, whereas caspase-6 truncates Tau at D13, D402 and D421.¹⁷¹ It has also been demonstrated that activation of calpain-1 (μ -calpain) stimulated by A β 1-40 aggregates leads to the formation of a 17-kDa N-terminal fragment of Tau (Tau 45-230, 2N4R) which can induce cell death in neurons.¹⁷² Another neurotoxic N-terminal fragment of Tau corresponds to residues 26-230. An elevated level of this fragment occurs during neuronal apoptosis and is linked to the activation of caspases (calpains may also be involved).¹⁷³ It has also been found that 26-28 kDa N-terminal proteolytic fragments of Tau are present in the CSF of AD patients.¹⁷⁴ Conversely, calpain-1 has been implicated in generating Tau-CTF24 which is a 24 kDa C-terminal fragment of Tau cleaved at residue R242.¹⁷⁵ Tau-CTF24 encompasses the repeat region and lacks the N-terminal part of Tau. This fragment exhibits enhanced susceptibility to undergo heparin-induced aggregation and does not promote microtubule assembly. An age-dependent increase in the level of Tau-CTF24 was found in a mouse model of tauopathy and this fragment is also present in the human brains affected by tauopathies.¹⁷⁵ Additionally, some matrix-metalloproteinases (e.g., MMP-9) may generate Tau fragments with enhanced propensity to form oligomers which can be internalized by neurons.¹⁷⁶ Zhang et al. reported that the proteolytic processing of Tau by asparagine endopeptidase (AEP), a lysosomal cysteine protease, at residues N255 and N368, also leads to the formation of aggregation-prone fragments which presumably may trigger neurodegeneration.¹²⁷ Accordingly, the Tau fragment cleaved at N368 has been found in AD patients together with increased activity of AEP. The activity of AEP generates multiple Tau fragments including Tau256-368 which represents a major part of the repeat region. Tau256-368 exhibits strong ability to assemble into PHFs during heparin-induced amyloidogenesis *in vitro*. Two other AEP-generated fragments, Tau1-368 and Tau256-441, also form typical PHF structures. This contrast with the case of amyloid fibrils formed by Tau1-255 and Tau369-441, where PHF morphology has not been observed. Notably, Tau1-368 and Tau256-368 have stronger ability to trigger apoptosis in neurons than the other proteolytic fragments, which suggests that the removal of the C-terminal tail located after N368 residue leads to increased neurotoxicity.¹²⁷ The mechanisms by which diverse proteolytic fragments of Tau may lead to neurodegenerative changes are unclear. The most amyloidogenic proteolytic fragment of the molecule encompasses the repeat region ("the

PHF core”) that has the strong ability to fibrillate and form PHFs.¹²⁷ Clearly, many proteolytic fragments of Tau are characterized by increased susceptibility to form amyloidogenic and/or toxic intermediates. It has been reported that the truncated forms of Tau participate in the formation of NFTs.¹²⁹ Proteolytic processing together with hyperphosphorylation of Tau precede formation of NFTs,¹³¹ however, it is not clear which of these PTMs may occur first. Nonetheless, *in vitro* amyloidogenesis of proteolytic fragments of Tau usually requires polyanionic inducers, however some fragments can aggregate even in the absence of polyanions.¹⁷⁷ Proteolysis very often leads to the formation of Tau fragments representing the repeat region of Tau (R1-R4, the residues 244-368 of the longest Tau isoform). These fragments have increased propensity to aggregate both *in vitro* and in the brains. As a result, proteolysis which removes fragments flanking the repeat region may be an important mechanism promoting Tau amyloidogenesis. Proteolysis may also generate fragments of Tau whose aggregation is markedly enhanced by the exposition of hexapeptide motifs, PHF6 and PHF6*, which are also located within the repeat region.¹⁷⁸ Truncated Tau is found in PHFs. A 12 kDa Tau fragment of approximately 100 residues was identified as the minimal protease-resistant Tau sequence that represents the core of PHFs in AD.¹⁷⁹ Due to the fact that Tau cleaved at E391 (isoform 2N4R) is a component of PHFs in AD, it was proposed that truncation at this site may lead to Tau fibrillization into PHFs and subsequent formation of NFTs.^{179–181} To conclude, the proteolytic removal of the sequences flanking MBD provides short aggregation-prone fragments which can contribute to the progression of Tau pathology in the human brain.

Glycosylation of Tau

A growing body of literature indicates that apart from phosphorylation and proteolytic truncation other PTMs may be involved in Tau amyloidogenesis. Some of these PTMs influence Tau phosphorylation and thereby indirectly modify amyloidogenic properties of the molecule, whereas others may affect stability of PHFs. It has been reported that glycosylation of Tau may lead to opposite effects depending on the type of the modification. Aberrant *N*-glycosylation through *N*-linkage of glycans at asparagine residues stimulates Tau hyperphosphorylation at several sites catalyzed by some kinases, including PKA, cdk5 and GSK-3 β .¹²³ It has been reported that *N*-linked glycans can make Tau molecules more susceptible to hyperphosphorylation and less prone to dephosphorylation.¹⁸² It has been observed that deglycosylation of PHFs turns them into bundles composed of single fibrils, therefore it seems that *N*-linked glycans may promote formation and stabilization of PHFs by maintaining their helicity.¹⁸³ Consequently, a possible

mechanism explaining the role of *N*-glycosylation in Tau amyloidogenesis relies on both stimulation of Tau hyperphosphorylation and stabilization of PHF conformation. Conversely, *O*-GlcNAcylation—a type of *O*-glycosylation—has been proposed to inhibit Tau phosphorylation which could eventually protect Tau from hyperphosphorylation in AD.^{184–186} Glycosylation through *O*-linked glycans at S/T sites blocks hydroxyl groups of these residues.¹²³ This prevents their phosphorylation and is likely to inhibit Tau amyloidogenesis. Consistently with that, elevated levels of *N*-glycosylation and decreased levels of *O*-GlcNAcylation of Tau have been detected in AD brains.^{123,187}

Acetylation of Tau

Another modification of Tau, namely lysine acetylation precedes formation of NFTs, prevents degradation of hyperphosphorylated Tau and also directly enhances its aggregation *in vitro*.^{133,134} Tau acetylated at K174, K274, K280, and K281 has been found in the brains of patients with AD.^{133,135,136} Furthermore, amyloid aggregates of Tau specifically acetylated at K280, that is located within the PHF6* motif of the repeat region, have been found in the brains of patients with AD and related tauopathies.^{188,189} It has been proposed that acetylation of Tau at K280 may lead to dissociation of Tau from MTs.¹³³ Subsequently, acetylated Tau may be released from MTs and becomes prone to other PTMs and fibrillization. Increased levels of acetylation are found in PHFs.¹³³ In a heparin-induced Tau fibrillization assay, both acetylated monomers of full-length Tau (2N4R) and K18 show an enhanced ability to form fibrils.¹³³ A possible mechanism that explains the role of Tau acetylation in its amyloidogenesis is based on neutralization of charges within the repeat region, which also decreases the binding of Tau to MTs.¹³⁴ In particular, hyperphosphorylation may neutralize remaining positive charges on the hyperacetylated Tau.

Prolyl-isomerization of Tau

Another enzyme which can influence the aggregation of Tau is peptidyl-prolyl *cis/trans*-isomerase (Pin1) catalyzing prolyl-isomerization. It has been found that Tau phosphorylated at a threonine residue of the T231-P motif may exist in *cis* and *trans* conformations.¹³⁷ The *cis* conformer is resistant to dephosphorylation and is prone to aggregation, and is believed to be an early precursor of Tau pathology.^{190,191} It has been proposed that Pin1 is involved in the protection against Tau aggregation in AD by isomerizing the phosphorylated molecule from *cis* to *trans* conformation.^{137,190} Furthermore, Pin1 is required for dephosphorylation of Tau.¹⁹⁰

SUMOylation and ubiquitination of Tau

PTMs which are involved in degradation pathways of amyloid aggregates of Tau include SUMOylation

and ubiquitination. These two PTMs may display opposite roles in Tau amyloidogenesis. SUMOylation has been shown to inhibit the ubiquitination of Tau, which may contribute to reduced degradation and increased accumulation of aggregated Tau.¹³⁹ Due to the fact that ubiquitination has been implicated in the targeting of aggregated species of Tau for degradation in the UPS^{140,192} its inhibition by Tau SUMOylation may result in enhanced accumulation of Tau amyloid aggregates in neurodegenerative conditions. In AD brains, Tau has been found to be ubiquitinated at three lysine residues: K254, K311, and K353.^{140,141} So far, there is evidence for Tau SUMOylation at only one lysine residue—K340.¹³⁹ Interestingly, SUMOylation stimulates Tau phosphorylation and vice versa.¹³⁹ It has been proposed that SUMOylation may induce conformational changes in Tau which make it a better substrate for protein kinases.¹³⁹

Methylation of Tau

Methylation of Tau at lysine residues has been found both in normal and AD brains. The precise role of methylation in Tau biology is, however, unclear. Tau in PHFs derived from AD brains has been found to be methylated at K44, K163, K174, K180, K254, K267, and K290.¹⁴² Conversely, Tau methylation also occurs in normal human brains.¹⁴³ Since methylation very often occurs within MTBRs (seven lysine residues in this region were found to be methylated), it is possible that it may influence affinity of Tau to MTs and affect the amyloidogenesis. Methylation may inhibit Thiazine-red-induced fibrillization of Tau by affecting nucleation and elongation phases as well as maturation of Tau fibrils.¹⁴³ Lysine residues in Tau, which can be methylated, overlap with some sites that can be acetylated, ubiquitinated, as well as glycosylated and polyaminated (Table I). Moreover, methylation of Tau may influence its susceptibility for phosphorylation and proteolytic cleavage.¹⁴²

Polyamination

Activity of transglutaminases (TGs) may lead to incorporation of polyamines (polyamination) into proteins. TGs may also catalyze formation of ϵ -(γ -glutamyl) lysine isopeptide bonds (either inter- or intramolecular) involving glutamine and lysine residues.^{144,193} Transglutaminase-catalyzed bonds are found in PHFs and NFTs in AD¹⁹⁴ and PSP brains.¹⁹⁵ It has been demonstrated that polyaminated Tau has increased stability and is more resistant to calpain-mediated proteolysis.¹⁹⁶ Polyamination has not been shown to affect the ability of Tau to bind MTs¹⁹⁶ or stimulate fibrillization.¹⁹³ The molecular mechanisms by which TGs may contribute to Tau amyloidogenesis are unclear. However, it has been proposed that TGs

can link Tau molecules with each other or with some other proteins that are detected in NFTs.¹⁴⁴

Nonenzymatic PTMs of Tau

Tau undergoes also nonenzymatic PTMs, including glycation and nitration. It has been reported that glycation can enhance amyloidogenesis of Tau *in vitro*.¹⁹⁷ This PTM does not promote the nucleation phase,¹⁹⁷ but can stabilize Tau amyloid fibrils most likely through cross-linking of Tau molecules.¹⁹⁸ Indeed, PHFs are found to be glycosylated in AD, whereas soluble Tau derived from AD brains is not glycosylated.¹⁹⁸ *In vitro*, glycation promotes aggregation of AD-derived Tau into large tangle-like aggregates formed by PHFs, which correlates with an increased ability of hyperglycosylated Tau to sediment.¹⁹⁸ Glycosylated recombinant Tau (obtained by incubating Tau with glucose) is more prone to form amyloid fibrils in an inducer-mediated fibrillization assay than the unmodified protein.¹⁹⁷ On the contrary, it has been reported that another nonenzymatic modification—nitration inhibits formation of Tau amyloid fibrils,¹⁴⁷ but stimulates Tau oligomerization *in vitro*.¹⁹⁹ Plausibly, nitration may also influence Tau amyloidogenesis in AD and other tauopathies.¹⁴⁷ Tau has been shown to be nitrated at five tyrosine residues: Y18, Y29, Y197, Y310, Y394.^{146,147} Nitration at Y18, Y29, Y197, and Y394 has been found in AD brains.^{146,147} A molecular mechanism which could explain how nitration may affect Tau amyloidogenesis remains unclear.

Characterization of Oligomers and Amyloid Fibrils of Tau

Tau oligomers

As in the case of other amyloidogenic proteins, oligomeric species of Tau are formed transiently during amyloidogenesis and represent highly heterogeneous group of assemblies. It is unclear whether oligomers are on- or off-pathway species. It has been suggested that Tau oligomers may be converted into amyloid fibrils.²⁰⁰ Oligomers are considered to be significantly more toxic than mature fibrils. One of the postulated mechanisms of cytotoxicity of oligomers involves disruption of integrity of biological membranes through permeabilization of lipid bilayers.²⁰¹ Oligomer-membrane interactions may lead to the formation of structures that resemble ion channels.²⁰² The oligomers may also interact with other proteins which may lead to the failure of PQC mechanisms and propagation of misfolded proteins within the cell.²⁰³ Interestingly, it has been shown that Tau oligomers may appear as a result of traumatic brain injury,²⁰⁴ hyperthermia²⁰⁵ and aging.²⁰⁶ The oligomeric assemblies of Tau have been reported to cause mitochondrial and synaptic dysfunctions,²⁰⁷ inhibit long term potentiation (LTP)²⁰⁸ and disrupt memory

Figure 3. Amyloid fibrils of Tau visualized by TEM. Recombinant human Tau forms polymorphic fibrils *in vitro*. These assemblies may resemble amyloid fibrils found in tauopathies. **(A)** SFs formed by recombinant human full-length Tau (2N4R, 441 amino acids). **(B)** PHFs formed by the K18 peptide. The scale bar is 100 nm

in animal models.²⁰⁹ Conversely, oligomeric species of Tau lacking the capacity to cause pathological changes have been reported, as well.²¹⁰ Tau oligomerization can also be influenced by other amyloidogenic proteins via direct binding. For example, the interaction between Tau273-284 (a fragment within the second repeat of MTBR encompassing PHF6*) and A β 25-35 (an amphipathic fragment of A β peptide which retains significant ability to aggregate) may lead to the formation of “heterologomers” (oligomers made of these two peptides).²¹¹

Tau amyloid fibrils

In recent years, numerous studies have discussed the role of amyloid fibrils in the pathology of neurodegenerative diseases, suggesting that they may be less toxic than the oligomers.^{203,212,213} However, there are numerous contradictory reports suggesting that amyloid fibrils of Tau may lead to propagation of amyloid pathology within the brain.^{17,22,214,215} It seems plausible that Tau fibrils contribute to the pathology that may be initiated by Tau oligomers at the earlier stages of amyloidogenesis.

As mentioned above, the typical forms of Tau amyloid in AD are PHFs (~95%), whereas SFs are less frequent.^{216–218} AD-derived PHFs are made of all six Tau isoforms.²¹⁸ These assemblies exhibit helical symmetry, twisted, right handed structure with a periodicity of ~80 nm and a width of ~20 nm.^{217,219} On the basis of the observation that *in vitro*-generated single fibrils of Tau may pair and twist together over incubation period, it has been proposed that SFs may be converted into PHFs.²²⁰ Fibrillar species of Tau aggregates have a protease resistant core composed of the microtubule binding repeats.^{179,221} It has been proposed that this region is also involved in association of PHFs into NFTs through lateral interactions between filaments which may locally expose their cores.²²² This is

supported by observation that *in vitro*-generated PHFs associate into bundles upon treatment with trypsin, which digests N- and C-terminal parts of Tau amyloid leaving the amyloid core.²²² Other proteases can also degrade susceptible components of Tau amyloid leaving the resistant regions.²²³ Interestingly, PHF morphology has also been reported for *in vitro*-generated fibrils assembled from the repeated domain of Tau (K18 peptide) (Fig. 3).

On the contrary, SFs represent a minor population of Tau aggregates found in AD and do not exhibit helical morphology and periodicity.²¹⁸ Hybrid fibrils in which single straight filaments change into PHFs (the transition occurs within a single fibril) can also be found in AD brains.²¹⁷ These observations lead to the conclusion that amyloid fibrils of Tau do not constitute a homogenous population, but are polymorphic in the brains of AD patients. In FTDP-17, Tau fibrils made of 3R and/or 4R Tau are predominantly single, but can also appear as PHFs, twisted ribbons and rope-like fibrils.²²⁴ In CBD which is known as a 4R tauopathy,²²⁵ Tau aggregates into SFs that are ~15 nm wide as well as PHFs that are ~26–28 nm in their maximal widths and have ~200 nm intervals.²²² In PiD which is a 3R tauopathy,²²⁶ Tau assembles into SFs that are ~15 nm in width and also into PHFs that are ~26 nm in width and exhibit periodicity of ~120 nm.²²⁷ These findings prove that structure of Tau filaments may differ between AD and non-AD tauopathies in morphological features. *In vitro*, the 3R isoforms have tendency to form twisted fibrils, whereas the 4R isoforms preferentially aggregate into single amyloid assemblies.⁹¹ It has also been proposed that structural polymorphism of fibrils assembled from 3R and 4R Tau may be influenced by the formation of a disulfide bond (either intermolecular in 3R Tau or intramolecular in 4R Tau).⁹⁰ It has been demonstrated that *in vitro*-generated Tau

fibrils share some common characteristics but may also differ in thickness, ability to form twisted or untwisted structures and periodicity.²²⁸ Moreover, structural heterogeneity often occurs in a single sample that contains fibrils assembled from identical Tau molecules.

Spectroscopic analysis has brought contradictory data on the content of secondary structures of Tau molecules in the amyloid assemblies. Some groups postulated that Tau amyloid fibrils are β -sheet rich,^{229,230} whereas others suggested lack of cross- β structure and an unusual α -helical architecture.²³¹ The characterization of Tau aggregates based on IR-spectroscopy has not revealed unambiguously the question as to whether amyloid fibrils have structured cores covered with unstructured coats, or fully structured fibrils are mixed with disordered protein molecules. Nevertheless, numerous observations support β -sheet rich nature of Tau amyloid. Fibrils formed from K18 or K19 polypeptides also have increased content of β -sheets.⁷³ K18 carrying mutations of FTDP-17 has been shown to have even more noticeable content of this secondary structure.⁷³ As mentioned, VQIINK and VQIVYK are nucleating segments of Tau that are considered to initiate formation of β -sheet conformation. VYK (located within the PHF6 motif) is the shortest Tau-derived peptide able to form amyloid fibrils.²³²

Full-length Tau and short polypeptides of Tau representing MTBRs can form fibrils of similar thickness as visualized by TEM and AFM.²²⁸ Consequently, it has been proposed that, besides a rigid β -sheet rich core made of the repeat region, a major part of Tau molecule in fibrils is undetectable by microscopy. This “invisible” component is known as the fuzzy coat. It is a disordered negatively-charged two-layered polyelectrolyte brush composed of the N-terminal and C-terminal regions of Tau.²²³ High-resolution AFM in the force-volume mode (FV-AFM) provided evidence that the fuzzy coat is an important integral element of Tau fibrils composed of the full-length protein.²²³ The structural characteristics of the fuzzy coat may be influenced by changes in pH and electrolyte concentration.²²³ Moreover, stability of the fuzzy coat may be regulated by hyperphosphorylation of Tau molecules.²³³

Cross-Seeding in Tau Amyloidogenesis

It has been proposed that cross-seeding between Tau and other amyloidogenic proteins may lead to the emergence of novel amyloid assemblies that exhibit unique properties. In cross-seeding, preformed amyloid seeds of one protein interact directly with singly dispersed (e.g., in solution) “native” molecules of another yet, sufficiently similar protein causing the latter to convert into amyloid fibrils as well.^{234–236} One of the key conditions of cross-seeding to occur is that the specific conformation of the seed needs to be

compatible (i.e., energetically allowed) with the protein being recruited (seeded). In other words, there must be an overlap in the configurational spaces of both proteins corresponding to the same amyloidogenic self-assembly pattern. Such a pattern (i.e., particular structural variant of amyloid fibril) does not need to be thermodynamically preferred (i.e., most stable) among all amyloid polymorphs accessible to either protein. It is therefore sufficient that molecular docking of monomers at the tip of a structurally-compatible amyloid seed opens a transition pathway toward amyloid that is faster than *de novo* aggregation of these monomers, for the cross-seeding to take place. In other words, the seed added to dispersed monomers provides a transition shortcut to amyloid structure essentially identical, or very similar to the seed itself. This can manifest in the form of so-called conformational memory effect when daughter fibrils obtained through cross-seeding follow, with a high fidelity, the biophysical, biochemical, and structural characteristics of the mother seed, rather than the type of amyloid that would be formed through spontaneous (unseeded) aggregation. Hence, cross-seeding is likely to lead to thermodynamically “frustrated” fibrils which nevertheless remain kinetically stable on the time-scales of cell life and of typical experiments carried out *in vitro*. It should be stressed, however, that sheer acceleration of aggregation of one protein by amyloid seeds formed by another (either *in vivo* or *in vitro*) may also be a consequence of less specific molecular interactions (e.g., secondary nucleation) which would not warrant the exact structural correspondence between the seed and daughter fibrils. Hence, in principle, we may talk about effective “cross-seeding” without “conformational memory effect”. Cross-seeding is either bidirectional (one protein seeds another and vice versa) or unidirectional (one protein seeds another, but it does not happen in the opposite way). However, protein interactions may sometimes lead to the cross-inhibition of misfolding.²³⁴

Cross-seeding may be implicated in certain mechanisms of pathogenesis in neurodegenerative diseases since co-deposition of amyloid aggregates of distinct proteins and peptides is frequently observed in these disorders. In particular, both Tau and A β amyloid assemblies accumulate in AD brain. It has been observed that A β aggregates accelerate Tau fibrillization²³⁷ and can force Tau to form PHFs.²³⁸ Tau interacts with the central to C-terminal regions of A β peptide.²³⁹ The R2 repeat in Tau is involved in direct interaction with A β 17–42 oligomers.²⁴⁰ Cross-breeding of the mice which express P301L Tau with the mice expressing mutant hAPP leads to enhanced assembly of NFTs, whereas formation of amyloid plaques is not accelerated.²⁴¹ Furthermore, injection of A β 1–42 fibrils into the brains of P301L Tau mice enhances formation of NFTs.²⁴² Based on these

observations, cross-seeding between Tau and A β has been proposed to be unidirectional.²³⁴ It has been reported that A β 1–42 hexamer protofibrils can bind to K18 and K19. Tau polypeptides cross-seeded by A β may adopt more extended and destabilized conformations which expose their hexapeptide motifs (PHF6 and PHF6*²⁴³). Cross-seeding between Tau and A β has been proposed to proceed through a “stretching-and-packing” mechanism.²⁴³

Jensen et al. have demonstrated that Tau interacts with C-terminal part of α -synuclein molecule through the microtubule-binding region.²⁴⁴ Subsequently, Tau has been shown to undergo cross-seeding with α -synuclein fibrils. Such seeding leads to intracellular accumulation of hyperphosphorylated Tau amyloid aggregates.²⁴⁵ Moreover, distinct strains of α -synuclein amyloid aggregates may be involved in cross-seeding of Tau.²³ In addition, amyloid aggregates of α -synuclein have been suggested to be involved in AD pathogenesis.²⁴⁶ It has been proposed that co-aggregation of Tau and α -synuclein in neurodegenerative diseases is bidirectional. Interestingly, α -synuclein has been reported to inhibit A β deposition and plaque formation.²⁴⁷ Consequently, understanding the complex relationships between aggregation processes of Tau, α -synuclein and A β in pathogenesis of AD may provide important insights into the molecular mechanisms of this disease.

It has also been postulated that interactions between Tau and huntingtin may influence progression of Huntington’s disease (HD). An increased level of expression of 4R Tau isoforms was detected in HD brains in which Tau forms nuclear rod-like deposits.²⁴⁸ Moreover, Tau is hyperphosphorylated, prone to oligomerization and forms pathological deposits which co-localize with the aggregates of mutant huntingtin in HD brains.²⁴⁹ Interestingly, expression of HD-linked mutant of huntingtin has been demonstrated to induce Tau hyperphosphorylation and interaction of intracellular huntingtin inclusions with Tau may lead to the formation of huntingtin/Tau inclusions of ring-like morphology.²⁵⁰

There are also numerous reports showing that pathological deposits of prion protein aggregates in TSEs may be accompanied by Tau pathology. Aggregates of hyperphosphorylated Tau have been detected in patients with familial and sporadic forms of TSEs,¹⁵² as well as in animals experimentally infected with prion diseases.²⁵¹ Coexistence of prion protein amyloid aggregates and NFTs composed of PHFs was documented in some cases of GSS.²⁵² In addition, Tau was found to be abnormally phosphorylated in CJD.^{152,253} Tau interacts both with PrP^C and PrP^{Sc}.²⁵⁴ Furthermore, some mutants of prion protein exhibit an increased capacity to interact with Tau.²⁵⁵ Due to simultaneous pathology of prion protein and Tau in several TSEs, it is plausible that cross-seeding may also contribute to the

pathomechanisms of prion diseases. It appears likely that cross-aggregation provides an important source of polymorphic amyloid assemblies which may, for example, exhibit different patterns of spread through distinct brain regions. It remains to be elucidated to what extent cross-seeding and simultaneous deposition of amyloid aggregates of distinct proteins modulates pathology of tauopathies.

Conformational Memory in Tau Amyloidogenesis

It has been demonstrated that Tau amyloid aggregates isolated from human brains are able to recruit and convert soluble Tau monomers, triggering pathology in the cells and animal models of tauopathies.^{17,256} It is not only remarkable that such experimental transmission of tauopathy is possible, but also intriguing that the amyloid structures that emerge upon trans-organismal seeding acquire conformational traits of the amyloid seeds derived from disease-affected brains. It is tempting to rationalize this outcome in terms of the conformational memory effect in which daughter amyloid, self-assembling from the pool of seeded monomers, acquires structural characteristics of the template (seed).^{257–259} Such a process of molecular imprinting would parallel other cases of self-propagation of pathological traits recognized in several neurodegenerative diseases associated with protein misfolding. For example, similar seed-dependent processes may explain propagation of prions in TSEs. Consequently, mechanisms associated with the template-based conversion of PrP^C into PrP^{Sc} may explain the propagation of pathological amyloid aggregates in neurodegenerative disorders.^{28,224,260,261}

As we stated earlier, seed-controlled proliferation of conformational traits of amyloid aggregates may proceed even if the conditions of aggregation favor alternative amyloid structures. The self-propagating polymorphism of amyloid fibrils is therefore a generic feature of amyloidogenic proteins^{25,258,262} and should be accessible to Tau, as well. It has been reported that PHFs isolated from AD brains can seed fibrillization of recombinant human 0N4R Tau. Upon such seeding, structural characteristics of PHFs are passed on the nascent amyloid aggregates of recombinant Tau.²⁶³ Interestingly, in this study recombinant Tau lacking PTMs (e.g., phosphorylation) was able to adopt conformational features of AD-derived PHFs. This finding supports the hypothesis that amyloid fibrils formed during neurodegenerative conditions contain the information that can be passed on to native protein molecules. Such prion-like molecular copying leads to the replication of a pathological structure. The conformational memory effect has also been demonstrated in the case of Tau peptides comprising the repeat region. It has been found that while seeds of

K18 efficiently template aggregation of K18 monomers, they are not able to seed aggregation of K19 monomers.^{264,265} K19 seeds promote seeded-aggregation of both K19 and K18 which implies the existence of an asymmetric barrier in seeding between K18 and K19. Surprisingly, fibrils obtained by seeding of K18 monomers with K19 seeds acquire ability to seed K19 even after multiple cycles of seeding, suggesting that K18 is able to form fibrils with specific templating abilities that are able to propagate conformational features.²⁶⁴ However, templating effects in seeded amyloidogenesis of Tau may occur with somehow compromised fidelity. Meyer et al. have reported that amyloid fibrils assembled from K18 molecules are heterogeneous and may convert into another polymorph even in repeated cycles of homologous seeding, whereas K19 forms homogeneous fibrils which are not prone to undergo structural changes upon seed-dependent fibrillization.²⁶⁶ It is possible that the structural changes observed upon seed-based aggregation of Tau may appear due to, as yet, poorly understood limitations in templating and propagation of Tau amyloid assemblies.²⁶⁷ In principle, changes in the structure of Tau fibrils could be influenced by “structural drift” in which a given conformation of a fibril could evolve into a novel amyloid structure.²⁶⁸ Notably, some kind of “conformational evolution” may also occur during propagation of bona fide prions which have been proposed to exist as an ensemble of several conformations.^{269–271} A particular conformer may be prone to propagate in one organism, whereas in the other host its replication might face “the seeding barrier”, favoring amplification of another conformer. Importantly, there are a couple of reasons for phenotypic instability of certain strains. For example, heterogeneity of an amyloid population may reveal itself through a conformational drift observed upon prolonged self-propagation. An amyloid strain with a kinetic advantage over other amyloid variants will ultimately “win the race” in spreading its structural characteristics. This may change depending on the conditions of the seeding (Fig. 4). There are other possible pathways which could explain conformational drift of amyloid samples involving conformational switching, secondary nucleation and deformed templating.^{271,272}

It has been demonstrated that not all Tau mutants linked to FTDP-17 can seed wild-type Tau, implying that distinct Tau seeds have diverse seeding potencies. In particular, full-length Tau (isoform 0N4R) has been shown to be seeded by amyloid seeds of Tau mutant R406W but not by seeds of P301L mutant, in heparin-induced fibrillization.²⁷³ It is unclear whether such seeding barrier occurs as a result of sequence incompatibility or formation of a particular seeding-incompetent conformer. In addition, the peptide PHF6 does not possess ability to

seed longer Tau peptides.²⁷⁴ Moreover, PHF43 peptide (43-residue peptide encompassing the third repeat of Tau and flanking residues, N265-E338, devoid of R2 = V275-S304) forms single thin untwisted fibrils which can seed aggregation of the shortest isoform of Tau (0N3R). Interestingly, Tau (0N3R) fibrils assembled in the presence of seeds of PHF43 acquire typical PHF morphology.⁹³ Furthermore, fibrillization of Tau 0N3R induced by heparin is manifested by very slow kinetics of aggregation, whereas PHF43 aggregates rapidly. Incubation of Tau 0N3R with amyloid seeds of PHF43 results in significant acceleration of fibrillization.⁹³ Examples mentioned above indicate that the seeded aggregation of Tau resembles the prion-like process which may, however, exhibit unique features with respect to the templating abilities.

It has been frequently proposed that the progression of Tau pathology is a prion-like process in which seeded aggregation of normal Tau molecules by pathological seeds leads to the self-propagation of Tau amyloid aggregates within the brain.^{17,22,28,275} Distinct phenotypes observed in tauopathies and heterogeneity of these diseases have been linked with the phenomenon resembling prion strains.^{17,22} Sanders et al. have demonstrated that HEK293 cells expressing Tau244-372 accumulate intracellular deposits of Tau aggregates upon treatment with Tau fibrils.²² Injection of lysates of such cells into hippocampi of mice overexpressing Tau mutant P301S caused induction of Tau pathology. Such Tau aggregates can be transferred upon passage through several generations of P301S mice, maintaining their characteristics and the ability to cause brain pathology. Importantly, the inoculation was inefficient in wild-type mice. It has also been demonstrated that brain homogenates from distinct human tauopathies including AD, AGD, CBD, PiD, and PSP induce specific phenotypes in HEK293 cells.²² Kaufman et al. have isolated 18 distinct Tau amyloid strains from diverse sources.¹⁷ Upon treatment of HEK293 cells (expressing the repeat region of 2N4R Tau carrying P301L and V337M mutations) with these variants of Tau amyloids, distinct forms of deposits have been observed (diffused, mosaic, ordered, speckles, disordered, etc). These strains stably propagated and maintained their morphology in the cells during cell division. Moreover, such strains had different seeding activity, and more efficient seeding was associated with stronger toxic effects in the cells. After injection of cell lysates with selected strains into brains of transgenic mice, distinct levels of pathological changes in the brain regions specific for different strains were observed.¹⁷ The prion-like nature of Tau aggregates seems to be supported also by the observation that injection of brain extract from mice overexpressing Tau carrying mutation P301S into brains of mice overexpressing wild-type Tau leads to

Figure 4. Hypothetical pathways in seeded fibrillization of Tau leading to strains. Tau is able to form structurally distinct amyloid seeds, depending on aggregation conditions. In the upper (A) and middle (B) panels, two types of Tau seeds are presented as single filaments (assembled in condition A indicated in blue) or paired filaments (assembled in condition B indicated in grey). According to the conformational memory hypothesis, these seeds recruit Tau monomers (in green) and convert them into strains which are molecular copies of the maternal seeds even if aggregation environment favors formation of alternative amyloid assemblies. Alternatively, strains may “switch” via conformational transition in which one amyloid seed may induce formation of a strain which may have a different structure. In the bottom (C) panel, two types of seeds are presented in condition D (in brown) that favors assembly of seed D. Conformational drift hypothesis postulates that upon change of aggregation environment, a minor conformer (in this case seed C) may gain a selective advantage, replicating its conformation and dominating the population over time

the propagation of amyloid pathology within the brain.²⁷⁶ Synthetic Tau amyloid fibrils injected into the brains of mice expressing human P301L Tau induce neurodegenerative changes including hyperphosphorylation and aggregation of Tau, eventually leading to neuronal loss.²⁷⁷

It has been proposed that the spread of Tau aggregation occurs after internalization of Tau amyloid seeds from the extracellular milieu into the cytoplasm most probably through the endocytic pathway.^{278–280} The above-mentioned mechanism may explain typical progress of the pathology observed in tauopathies. It has also been demonstrated that the mice expressing human Tau mutant P301L exclusively in the entorhinal cortex and lacking endogenous (murine) Tau show trans-synaptic propagation of the mutated Tau in the dentate gyrus in the absence of the endogenous protein, which results in NFTs formation and spread of

neurodegeneration.²⁸¹ This seems to be in contrast to the case of TSE prions where both propagation and neurotoxicity are abolished in PrP-deficient mice. It has been shown that brain tissue lacking endogenous PrP^C is not infected with TSE or damaged after experimental inoculation with bona fide prions.²⁸²

Nevertheless, although the prion-like mechanism of propagation of amyloids is widely accepted, several recent studies have questioned its usefulness with respect to Tau-related pathologies.^{267,281,283} Differences in the propagation characteristic of Tau amyloid aggregates and bona fide prions can be explained by obvious differences in the biology of Tau and prion protein determined by the primary structure of the molecules. Moreover, in contrary to TSEs, tauopathies do not exhibit infectious etiology and there is no epidemiological data that would suggest transmissibility of these disorders between

humans.^{284–286} Taking into account these inconsistencies, some authors have proposed to classify amyloid aggregates of Tau into a group of “prionoids”.²⁸³

Conclusions and Future Directions

Tauopathies remain incurable neurodegenerative diseases in which Tau protein loses its principal function which is the stabilization of microtubular cytoskeleton. Molecular mechanisms of the conformational transitions and association of intrinsically unstructured Tau molecules into highly ordered amyloid assemblies, which are deposited in these diseases, remain challenging to investigate. Nevertheless, several factors have been shown to trigger conformational changes in Tau which may result in amyloidogenic events. These factors include mutations in *MAPT*, polyanions, several PTMs and cross-seeding by aggregates composed of other amyloidogenic proteins. The ability to undergo aggregation through seed-based autocatalytic pathway can liken Tau amyloids to prions. It seems likely that all of amyloid species of Tau can account for the propagation of pathological aggregates. Since Tau may interact with both A β and α -synuclein, studies on cross-seeding may be of wider importance due to its involvement in neurodegenerative processes. Possibly, cross-seeding may result in the generation of Tau amyloid polymorphs resembling amyloid strains.

Characterization of processes associated with Tau amyloidogenesis may provide novel targets for therapies that aim to cure tauopathies. Promising approaches have been focusing on targeting misprocessed Tau,²⁸⁷ inhibiting Tau hyperphosphorylation,^{288,289} stabilizing MTs by microtubule-binding drugs,²⁹⁰ blocking Tau seeding and trans-cellular propagation of Tau aggregates,^{214,291} and immunization with Tau oligomer-specific antibodies.¹¹ Nevertheless, to date, none of these approaches provided effective drugs or therapies. Future studies should focus on better understanding of molecular mechanisms underlying the emergence and the propagation of the amyloid state of Tau as well as the prevention of deleterious interactions between Tau and other amyloidogenic polypeptides.

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