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Chapter 8

TAU STRAINS AND THEIR PROPAGATION IN EXPERIMENTAL DISEASE MODELS

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ABSTRACT

Tauopathies encompass a broad family of neurodegenerative diseases, including Alzheimer's disease, which are characterized by the fibrillization of the microtubule-associated tau protein. The normal function of tau is to stabilize and promote the assembly of microtubules in neuronal axons. Sequestration of tau into amyloid fibrils results in destabilization of the microtubule network and may contribute to disease progression. As tau is an intracellular protein and proteins do not passively cross cell membranes, tau fibril formation has been assumed to occur spontaneously within individual cells. However, recent evidence suggests that tau shares several characteristics with prions, which propagate through the brain by protein-protein interactions in the interstitial space; these characteristics include conformational templating of native tau into disease-associated fibrils and intercellular fibril propagation. Tau adopts diverse fibril structures, or strains, which have been shown to self-propagate in the presence of monomeric recombinant tau protein. Exogenous tau fibrils induce misfolding of native tau in both cell culture and animal models, causing strain-dependent cellular dysfunction and differential patterns of neuropathology. Tau fibers have also been found recently in patient samples or models of several diseases not formerly identified as tauopathies, including chronic traumatic encephalopathy, Parkinson's disease, and Huntington's disease, suggesting a common underlying mechanism for neurodegenerative diseases. The possibility that tauopathies and other neurodegenerative diseases involve prion-like mechanisms has implications for studies designed to understand disease pathogenesis and for the development of therapies, which may be devised to impede tau strain propagation and intercellular transmission, allowing clearance of tau fibrils and potentially halting or reversing disease progression.

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INTRODUCTION

Misfolding of the microtubule-associated protein tau into deposits of neurofibrillary tangles is a distinguishing feature of a group of neurodegenerative diseases collectively referred to as tauopathies. Tauopathies are movement disorders and dementias that result in a progressive loss of neurons and cognitive impairment and include Alzheimer's disease (AD), Pick's disease, corticobasal degeneration, progressive supranuclear palsy, agyrophilic grain disease, chronic traumatic encephalopathy, and tangle-only dementia, among others [1]. The fibrillization and accumulation of tau is common among these diseases; however, the histopathological progression and clinical presentation differs between them [2], suggesting the possibility of distinct strains of tau fibrils with unique properties that would account for their stereotypic characteristics. Currently there are no reliable methods to diagnose patients with many of these diseases [3] and no therapeutics available to halt or even slow disease progression.

Native tau mainly functions by binding to and stabilizing neuronal microtubules to facilitate cellular structure and intracellular transport of biomolecules [4, 5] though tau may also play a role in many cellular pathways [6, 7]. In the unbound state, tau is an inherently disordered, highly soluble protein [8, 9]. The microtubule-binding region near the C-terminus reversibly associates with axonal microtubules in a phosphorylation-dependent manner [10]. There are 6 different splice variants or isoforms of tau (Figure 1) that are expressed in humans and their relative proportions in neurofibrillary tangles often varies among different tauopathies [11].

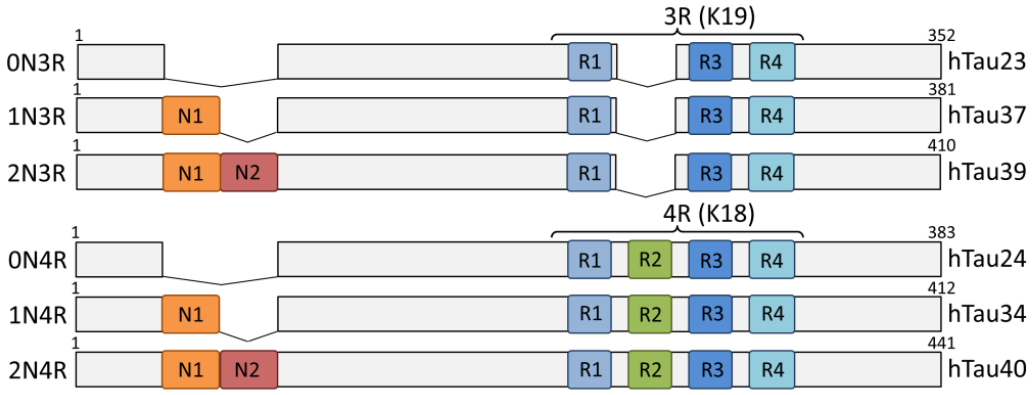

Figure 1. Splice variant isoforms of tau. The six different isoforms of tau generated by alternative splicing of exons 2 and 3 for the N-terminal region, which encode the N1 and N2 segments of the protein (labeled here in orange and red, respectively), and of exon 10 in the microtubule binding region, which encodes the second repeat sequence (R2, shown in green) [20]. Terminology used for describing the isoforms varies among researchers; the more descriptive names listed to the left of each isoform are used throughout this chapter. Historical names are listed to the right, along with the number of residues present in each isoform. The microtubule binding domain is composed of 3 or 4 repeat sequences (blue and green), which have significant homology but are not identical. These domains form the core of tau fibrils, and when expressed recombinantly as truncated protein products, they are referred to as 3R and 4R; alternately they are sometime identified as K19 and K18, respectively.

Alternative splicing produces an N-terminal region that may have zero, one, or two variable polypeptide segments while the microtubule-binding region may contain either three or four repeat sequences with moderate homology. Roughly equal amounts of 3R and 4R isoforms are found in fibrils isolated from the brains of patients with AD [12], chronic traumatic encephalopathy [13], and tangle-only dementia [14]. The 4R isoforms are predominant in fibrils found in progressive supranuclear palsy [15], corticobasal degeneration [16], and agyrophilic grain disease brain tissue [17, 18], while Pick's disease inclusions are predominantly composed of 3R isoforms [19].

Prions are infectious agents composed of alternatively folded protein with the ability to transmit disease through recruitment and conversion of normally folded protein into the alternatively folded conformation [21]; additionally, structurally distinct prion strains, composed of protein with identical polypeptide sequences, induce distinct phenotypic changes in host animals [22, 23]. The mechanism by which initial spontaneous misfolding of tau occurs is still not fully understood; however, increasing evidence suggests that propagation of fibrillar tau may be "prion-like" in its progression not only at the molecular level, but at the cellular level as well (Figure 2) [24, 25, 26, 27]. Neurodegenerative diseases in general appear to follow a pattern of neuronal connectivity in their progression [28, 29, 30] which may signify the propagation of a pathogenic species. Thus, the prion-like propagation of tau strains may provide an opportunity for developing therapeutics to prevent their intercellular spread.

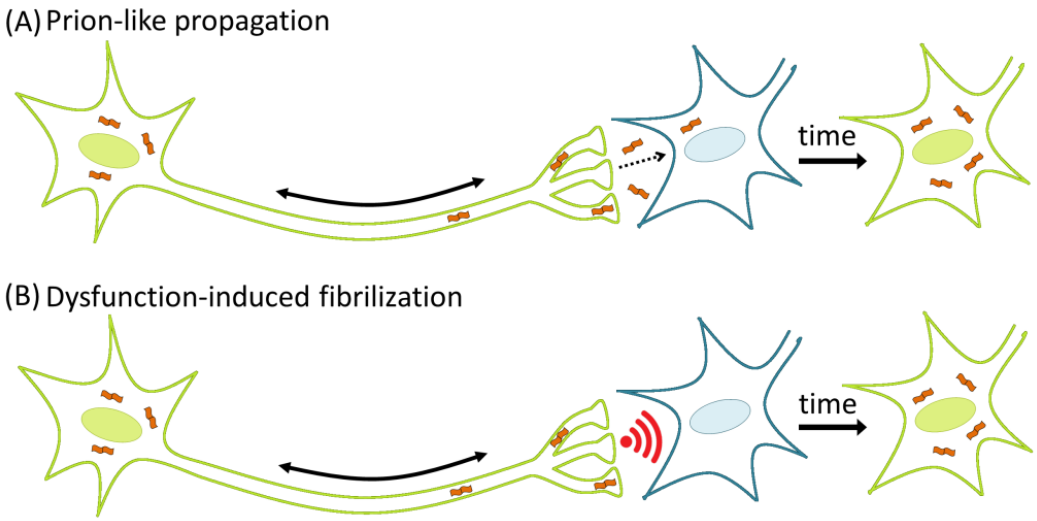

Figure 2. Competing hypotheses for the causative mechanism of disease pathogenesis. (A) The "prion-like" hypothesis of tauopathies suggests that strains of tau fibrils pass from dysfunctioning neurons (green) into healthy neurons (blue) and recruit native tau, resulting in dysfunction of the healthy neuron over time. (B) An alternate hypothesis is that dysfunctioning neurons induce a state of stress in healthy neurons through signaling, causing tau fibrillization as a downstream effect, e.g. through disruption of protein homeostasis or induction of apoptosis [31]; this hypothesis is akin to the amyloid cascade hypothesis in AD. The observation that tau strains are conserved throughout the brain as they spread supports the first hypothesis.

PRION-LIKE PHENOMENON IN TAUOPATHIES

Shortly after the discovery of prions, the most common tauopathy, Alzheimer's disease, was hypothesized to share a common underlying mechanism for pathogenesis [32]. While most tauopathies are sporadic in etiology [33], genetic forms of these diseases caused by destabilizing point mutations have been discovered (Figure 3). The fact that such mutations are sufficient to initiate neurodegeneration suggests that tau fibrillization can initiate disease pathogenesis [34, 35, 36].

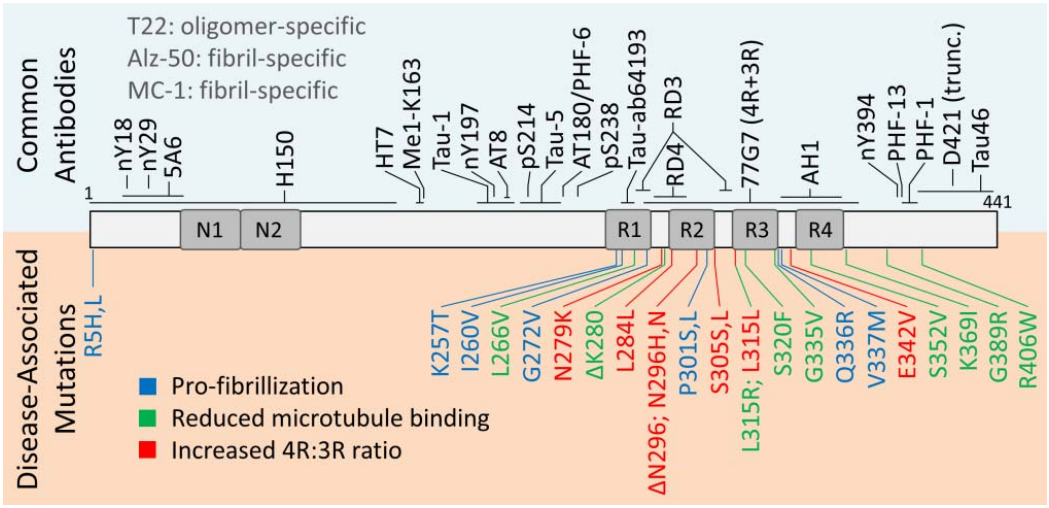

Figure 3. Commonly used tau antibodies and disease-associated mutations. (Top) Antibodies are mapped to the locations of antigens used to raise them, or to their epitopes if known. Antibodies T22, Alz-50, and MC1 are conformation-specific as indicated. The antibodies RD3 and RD4 are specific to the isoforms after which they are named. Several antibodies are specific for post-translational modification, including phosphorylation, nitration, and methylation. (Bottom) Disease-associated mutations are mapped to their locations on the gene. Mutations are associated with increasing fibril formation (blue), reducing the binding affinity for microtubule binding affinity (green), or increasing the 4R:3R splicing ratio (red). Some mutations such as L315L are silent gene mutations that effect tau mRNA splicing (Bottom updated and adapted from [36]).

The first extensive classification of the histopathological stages of AD demonstrated a distinct pattern of the spread of tau tangles in the brain along neuroanatomical connections [37], suggesting either protein-based disease progression or progressive cellular dysfunction in series of neurons (Figure 2). The hierarchical spread of tau pathology has also been documented in other tauopathies [38]. Templated conformational propagation of recombinant tau into structurally distinct strains [39,40], transynaptic spread of tau fibrils and pathology in mice [41, 42], and the spread of tau fibrils following inoculation of exogenous fibrils [43, 44] all provide supporting evidence for the prion-like spread of tauopathies (Table 1). Serial propagation of strains has also been demonstrated in mice [45, 46] analogous to that of prion inoculation [47].

One distinguishing feature of *bona fide* prions is their transmissibility from one individual to another. An epidemiological study of the prevalence of several neurological diseases associated with protein misfolding in patients receiving injections of human growth

hormone isolated from cadaver pituitary glands concluded that, with the exception of prion diseases, such diseases are not iatrogenically transmitted between patients in this fashion [48]. However, intraperitoneal injection of tau fibrils induced tauopathy in the brains of mice in another study [49]. Regardless of whether tauopathies are transmissible, such diseases do share key characteristics with prions at the molecular and cellular level.

Table 1. Summary of evidence for prion-like properties of tau strains

Model System	Evidence of strain-like behavior	References
Biochemical	Synthetic tau strains faithfully propagate using recombinant protein	[39]
	Conformational properties of both mouse and human brain-derived fibrils faithfully propagate using recombinant protein	[40, 77]
Cell Culture	Synthetic strains form unique puncta morphologies and retain biochemical properties	[46]
	Human brain-derived fibrils form unique puncta morphologies	[46]
Transgenic mice	Strains have differential patterns of neuropathology, including brain regions and cell types affected	[45, 46, 93]
	Tauopathy-derived strains exhibit histopathological features of their human counterparts when passaged to mice	[45, 93]
	Fibrils maintaine structural and biochemical features during three serial passages in mice	[46]

FORMATION OF SYNTHETIC TAU STRAINS FROM RECOMBINANT PROTEIN

Analogous to the diversity of conformational strains found in prion disease [22, 23, 50, 51, 52, 53], fibrils isolated from brains of patients diagnosed with different tauopathies also exhibit a range of conformations (Figure 4a) [54, 55, 56, 57]. Tau protein natively exists as an inherently disorganized structure consisting mostly of random coil, adopting a range of monomeric structures over a broad energy potential landscape [5, 58]. The energy potential landscapes of amyloidogenic proteins contain intermediate states which bind to amyloids, leading to elongation of fibrils [59, 60, 61]. This conversion is accompanied by a loss of random coil content and an increase in beta-sheet content [9, 60, 61].

Full-length tau produced recombinantly forms synthetic fibrils [62, 63], a process which is induced by polyanionic cofactors including sulfated glucosaminoglycans (*e.g.* heparin), RNA, or free fatty acids [64, 65, 66, 67]. Investigation of the protein regions responsible for driving synthetic fibril formation indicated that the third repeat domain and adjacent sequences are sufficient to form fibrils [68]. In the presence of polyanionic inducers, the mechanism of fibril formation follows nucleated monomer-addition kinetics [69, 70, 71, 72]. Such studies have provided insight into the molecular mechanism leading to spontaneous formation and stabilization of fibrils in the early pathogenesis of tauopathies.

Distinct fibril strains have been shown to propagate by sequestering the same recombinant tau substrate. Two different fibril conformations formed from wild-type and mutant tau in the presence of an inducer were subsequently used to seed monomeric wild-type tau; the tau structures formed maintained the structural characteristics of the original seeds [39]. Other studies have shown that two different induced fibrils from 4R tau isoforms had distinct properties, and only one of the conformations was able to seed a 3R tau substrate [73, 74]. In addition to potential roles played by genetic polymorphisms and splice variants, thiol-disulfide interactions may contribute to fibril diversity [75].

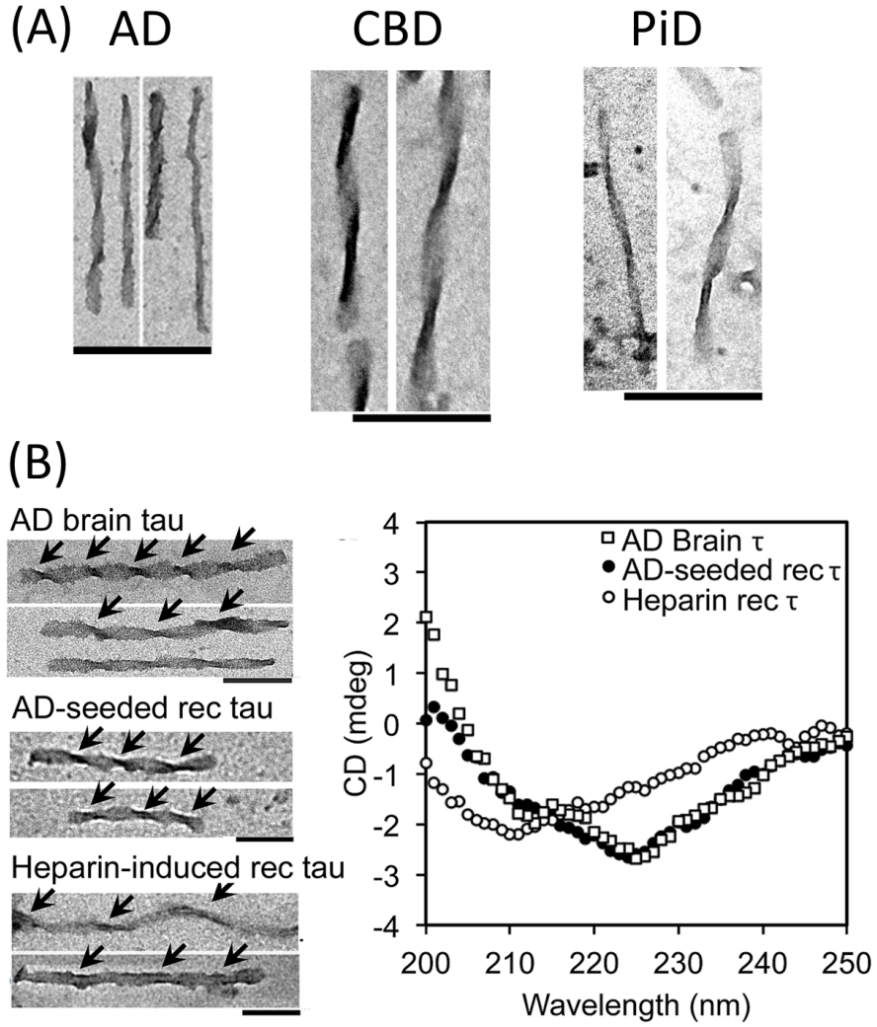

Figure 4. Tau strains and their propagation in cell-free systems without post-translational modification or cofactors. (A) Electron micrographs of the distinct structures of tau fibrils isolated from AD, corticobasal degeneration (CBD), and Pick's disease (PiD) brain homogenates. (B) Monomeric recombinant protein seeded with tau fibrils isolated from AD brain homogenates retains the structural features of the parental seed as assessed by electron microscopy and circular dichroism. For comparison, recombinant tau protein induced to form fibrils by heparin have distinct features. Scale bars represent 200 nm in (A) and 100 nm in (B); arrows indicate period of twist; images in (B) reprinted with permission from [40], copyright 2013 American Chemical Society.

SEEDED AMPLIFICATION OF BRAIN-DERIVED TAU STRAINS

While polyanionic fibril induction has enabled insights into disease processes using recombinant protein, the underlying mechanism of fibril formation in the presence of such inducers appears to follow a different fibrillization mechanism than most amyloidogenic proteins [76], and the fibril strains formed are both structurally and biochemically different from their brain-derived counterparts (Figure 4b) [40, 77]. In contrast to these synthetic tau strains, full-length wild-type tau seeded by fibrils isolated from AD brain without heparin faithfully inherited the seed properties including fibril dimensions and secondary structure, independent of phosphorylation (Figure 4b) [40]. Hyper-phosphorylated tau has a reduced ability to stabilize microtubules so it has been implicated in tauopathy pathogenesis, though its role in fibril formation is a subject of debate [40, 78, 79, 80].

Additionally, recombinant tau seeded by P301S mouse brain-derived fibrils had the seeding potency and biochemical characteristics of the parent brain-derived seeds with and without hyper-phosphorylation [77] confirming the potential for tau strains to template conformational change independently of post-translational modifications [40].

TAU FIBRIL UPTAKE AND STRAIN PROPAGATION IN CELL CULTURE

Cell culture models have been used to study the mechanisms by which tau fibrils become internalized, propagate, and transfer between cells. Synthetic tau fibrils, formed by misfolding tau in the presence of polyanionic cofactors, can be directly taken up by cells, seeding fibrillization of endogenous tau [81, 82, 83]. AD brain-derived tau also seeded intracellular fibrillization in HEK cells and SH-SY5Y cells [84]. Tau fibrils enter primary mouse neurons without lipid-based protein delivery reagents and stimulate conversion of endogenously expressed tau [26, 85, 86, 87].

Tau fibril strains also propagate in cell culture. Fibrils of 4R tau induced several biochemically and morphologically distinct synthetic tau strains in HEK cells [46]. These synthetic strains propagate in HEK cells as the cells divide and infect naïve HEK cells while maintaining the cellular and molecular properties characteristic of each strain. Tau fibrils purified from AD, agyrophilic grain disease, corticobasal degeneration, Pick's disease, and progressive supranuclear palsy brains elicited morphologically distinct patterns of aggregation in HEK cells [46]. Finally, tau fibers purified from end-stage P301S mouse brain seed conversion of tau in HEK293 cells about fifty times more efficiently than biochemically distinct, synthetic fibrils composed of recombinant tau with the same sequence [77].

TRANSMISSION OF TAUOPATHIES IN MICE

The tendency of tau fibrils to be taken up by neurons and recruit endogenous tau into newly formed fibrils has also been demonstrated in mouse models of tauopathies. One mouse model expresses P301S mutant full-length human tau and begins to spontaneously develop tau tangles and neurodegeneration [88]. Intracerebral injection of P301S mouse brain

homogenate into ALZ17 mice, a mouse model expressing a wild-type human tau [89], seeded fibrillization of tau in ALZ17 mouse brains [43]. Since the ALZ17 mice do not develop tauopathy in their lifetime, the P301S mouse brain homogenate was inferred to have contained a transmissible species. Likewise, an injection of end-stage P301S brain homogenate into presymptomatic P301S mice accelerated fibrillization before the P301S mice normally develop tauopathy [90].

The induction of tau pathology in mice by synthetic fibrils made from recombinantly expressed tau [44] further supports the protein-only hypothesis. A larger dose of synthetic fibrils made from P301L 4R tau seeded tau fibrillization in mice expressing full-length P301L tau and additionally demonstrated selective neuronal loss in the hippocampus relative to wild-type mice injected with the same quantity of induced fibrils [91]. In addition to the structural differences between fibrils from AD brains and synthetic fibrils induced by heparin [40], brain-derived and synthetic fibrils have different seeding potency in mouse models*:* enhanced seeding of P301S brain homogenate relative to heparin-induced fibrils was observed [77]; likewise, AD brain-derived seeds fibrillized tau in wild-type mouse brains more potently than heparin-induced fibrils [92].

Mice expressing mutant tau only in the entorhinal cortex induced tau fibrillization in distal, but synaptically connected regions of the brain [41, 42]. Exogenously administered fibrils consistently induce a time- and dose-dependent pattern of transynaptic spread in mice [44, 46, 90, 91]. Patient brain-homogenates from six different tauopathies seeded tau pathology similar to their human counterparts in mice expressing wild-type 2N4R human tau [45] except for Pick's disease in which tau fibrils were confined to the injection site. Pick's disease is a predominantly 3R isoform tauopathy, so there may be a seeding barrier between 3R and 4R tau isoforms [74, 82]. Brain homogenate from corticobasal degeneration and AD patients also induced similar histological progression in mice expressing full-length P301S tau; the number of hippocampal neurons decreased significantly in the AD cohort, but not in the corticobasal degeneration mice [93]. Two synthetic strains successfully propagated biochemical features after multiple sequential inoculations in mice [46]. Tau fibrillization was also propagated through serial inoculation of P301S mouse brain homogenates in ALZ17 mice, and brain homogenate from tangle-only dementia and agyrophilic grain disease patients in wild-type mice [45], although it was not clear whether the specific patterns of tau fibrillization were faithfully propagated as well. The intercellular transfer of tau fibrils in the brain and potential alternate routes of administration indicates that antibody therapies or vaccination may be an effective approach to treat or prevent tauopathies [94]; some groups have demonstrated success with antibodies capable of attenuating disease pathology [95, 96, 97].

MECHANISMS IMPLICATED IN CELL-TO-CELL TRANSFER OF TAU FIBRILS

Unlike disease proteins that are trafficked to the cell surface like prions or Aβ, tau is normally located in the cytosol and tau fibrils localize to the perinuclear space or somatodenritic compartment [98]. Monomeric tau is released into the interstitial space related to neuronal activity [99, 100] and is present in CSF [101]. It is unclear whether the release of

pathogenic tau from neurons is related to the normal pathway of physiological release or unconventional secretory mechanisms [102, 103].

Experimentally, tau fibrils have been demonstrated to transfer between cells in cell culture [81, 104, 105] and transynaptically *in vivo* [41, 42, 105, 106]. Transynaptic spread in mouse models and both anterograde and retrograde trafficking of misfolded tau in neurons [87] strongly indicates the importance of synaptic connectivity in tauopathy pathogenesis. Alternatively, tau may be released into the interstitial space by the death of neurons containing tau fibrils. However, tau spreads in a hierarchical pattern following synaptic connectivity to distal regions of the brain [37, 41, 42, 43, 44, 45]; moreover tau levels do not appear to correlate with lactate dehydrogenase or tubulin release [107, 108]. Therefore it is unclear whether cell death is a dominant mechanism of intercellular transfer.

The detailed steps by which pathogenic uptake of tau fibrils occurs are also not fully understood, although several potential mechanisms have been identified (Figure 5).

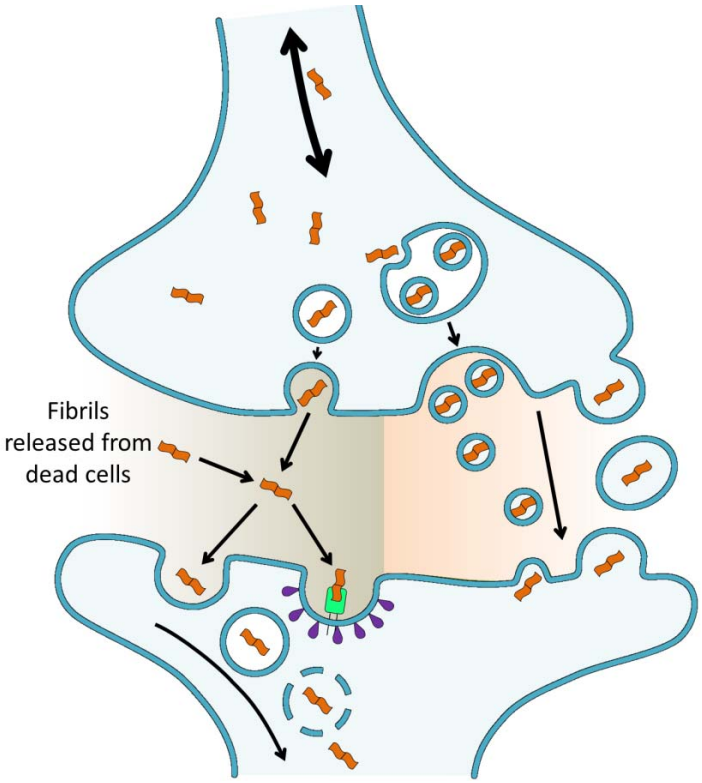

Figure 5. Hypotheses for the intercellular transfer of tau seeding species. Tau fibrils (orange) are transported both retrogradely and anterogradely along axons, as indicated by the large double-headed arrow at top, allowing fibrils to traverse neurons to presynaptic and postsynaptic termini [87]. Fibrillar tau has been observed outside of the cell in free form or in extracellular vesicles [102]. Shown to the left, naked fibrils may enter the extracellular space by release from dying cells or vesicular release by exocytosis potentially related to synaptic activity. Free fibrils may then be taken up by fluid-phase endocytosis or receptor-mediated endocytosis (receptor and vesicular coat proteins indicated in green and purple, respectively). Endosomal tau must then escape into the cytosol before it can recruit native tau into fibrils. Shown to the right, membrane-associated tau originates from exosomal/microvesicular packaging and release [113]. Once the membrane-associated tau reaches a post-synaptic terminal, it may be taken up by exosome/microvesicle fusion with the cell membrane.

Empirically, synthetic and brain-derived tau fibrils enter the cytosol of immortalized cell lines [81, 83, 84] and neurons in culture [85, 87, 109], and in mouse brains [43, 44, 45]; uptake also appears to be related to synaptic connections [37, 41, 42]. Lipid-based transfection reagents are used to promote fibril uptake [82, 83, 109]; however, studies specifically addressing the direct uptake of free tau fibrils in multiple cell types agree that a dominant mechanism is macropinocytosis [84, 86, 87]. Macropinocytosis is an actin-driven endocytic pathway in which the cell membrane encapsulates and pinches off a large intracellular vesicle containing extracellular fluid and local biomolecules [110]. Early work identifying markers of macropinocytosis seemed to suggest non-specific uptake [81, 84, 87]; however, a recent study showed tau fibrils actively stimulate macropinocytosis in a dose-dependent manner [86]. Once internalized into intracellular membrane vesicles, the endosomal escape of tau fibrils must occur before they can interact with endogenous tau. Heparin sulfate proteoglycans have been identified in neuritic plaques [111]; however, it was recently demonstrated that blocking cell surface heparin sulfate proteoglycan interactions strongly inhibits fibril uptake in culture and in mice [86] similar to the heparin-mediated pathways by which some bacteria and viruses enter cells [112]. Exosomal membrane fusion has also been suggested as a possible uptake mechanism [113]. The intercellular transfer of tau may also occur by a combination of these mechanisms [114], although it is not yet clear which is dominant in disease progression.

ROLE OF TAU FIBERS IN NEURONAL DYSFUNCTION AND DEATH

The contribution of the tau fibrillization process to neurodegeneration is unclear, as is that of the fibers themselves [115], with several lines of evidence questioning a direct contribution. Some degree of tau pathology was found in all individuals examined over age 75, increasing steadily with age, in the absence of cognitive impairment (n=26) [116]. Neuronal death in tauopathy patients greatly exceeds the number of neurofibrillary tangles accumulated [117].

Suppression of mutant tau expression, by administration of doxycycline to ‘Tet-Off’ transgenic mice expressing P301L or ΔK280 mutant human tau and after cognitive deficits and tau pathology have begun to develop, leads to improvement in cognitive function despite the persistence of neurofibrillary tangles [118, 119, 120]. Behavioral impairment and neuronal loss occur in some tauopathy animal models in the absence of detectable insoluble tau [121, 122, 123]. Mice expressing full length mutant tau exhibit neuronal dysfunction before the presence of detectable, mature neurofibrillary tangles [119, 124]; further, fibrils from end-stage P301S mice intracerebrally injected into mice expressing full-length human tau develop clear histological tau pathology without obvious neuronal loss [43, 90]. These findings must be taken into account in dissecting tau’s role in neurodegeneration.

The toxicity of tau fibrillization may be directly related to the prefibrillar conversion of tau rather than the presence of mature tau neurofibrillary tangles themselves. It has been hypothesized that destabilization of protein quality control systems by age-related dysfunction or external stress (as in chronic traumatic encephalopathy) by a stressor threshold effect [31] may allow this toxic conversion process to occur more rapidly or accumulate more efficiently,

making upregulation of protein chaperones a potential therapeutic target [125, 126, 127]. As tau also has a physiological role in many cellular pathways [6, 7], rapid sequestration of tau by fibril elongation may elicit dysfunction by a sudden loss-of-function of tau [128, 129].

Post-translational modifications including phosphorylation may not be required to template existing strains [40]; however, they may influence the spontaneous origin of strains or affect the interactions of tau in these cellular pathways to cause dysfunction [79, 80]. Tau-induced microglial activation [130] or receptor activation [131, 132] may indirectly cause neuronal dysfunction in surrounding cells (Figure 2b).

CONCLUSION

The identification of distinct fibril structures and evidence for propagation of strains in tauopathies provides a possible explanation for the divergent histological and clinical characteristics of tauopathies. Although significant progress has been made, additional research is needed to firmly establish the dominant mechanisms of neuronal release and uptake, the role of synaptic connections and activity on cellular transfer, cell type-specific vulnerability, and the conditions or cofactors responsible for the spontaneous origin of tau strains. Accumulating evidence suggests that tau fibrillization progresses within and between cells via templated conversion, similar to prions, Aβ, and other neurodegeneration-associated proteins [133, 134]; however, elucidation of strain-specific contributions to toxicity and cellular dysfunction is complicated by the possibility of mechanisms involving cell-autonomous effects and those arising from interactions with surrounding cells.

There are currently no therapies available to slow or halt the progression of tauopathies. Understanding the mechanisms by which tau strains propagate and cause or contribute to neurodegeneration will likely facilitate the discovery or design of therapies [135, 136, 137] or immunization strategies aimed at particular conformations of tau [138, 139, 140, 141, 142].

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