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Review

Functions and malfunctions of the tau proteins

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Abstract. The tau proteins belong to the family of microtubule-associated proteins. They are mainly expressed in neurons where they play major regulatory roles in the organization and integrity of the cytoskeleton network. Neurofibrillary changes of abnormally hyperphosphorylated tau are a key lesion in Alzheimer's disease and a number of other tauopathies. However, despite an ever-increasing body of data on the changes which tau undergoes in disease, its role regarding the fundamental disease process is still unclear. Moreover, conceptions of tau functions continue to evolve, which complicates an understanding of its role in the disease process. This review attempts to summarize data on the role of tau proteins in the context of both normal cellular function and dysfunction. Furthermore, we try to develop a mechanistic framework for the involvement of tau during the disease process. The review closes with a look towards various approaches to elucidate the functions and malfunctions of tau.

Key words. Tau; microtubule; neuronal polarity; Alzheimer's disease; tauopathy; hyperphosphorylation; cytoskeleton.

One of the characteristic features of a neuron is its structural and functional compartmentalization into an axonal and a somatodendritic compartment. This event, which is essential for establishing the intricate wiring of the nervous system, represents a remarkable example of morphological and biochemical differentiation. As unique processes projecting from neuronal cells, axons can traverse long distances to make synaptic connections with target cells. The formation of axons and dendrites involves complex intracellular structures and signaling networks that must provide support and regulation for the spatial and temporal control of process elongation and polarization. The cytoskeleton is the major intracellular determinant of neuronal morphology and provides the structural framework for this compartmentalization. The integrity of the cytoskeleton is clearly critical for the function and survival of neurons, and thus many neurodegenerative diseases are characterized by typical abnormalities in the neuronal cytoskeleton.

Microtubules are among the most prominent structural components found in growing and mature neuritic processes. As a class of proteins, microtubule-associated proteins or MAPs have been shown to play important roles in promoting the assembly of tubulin into microtubules and then maintaining their structural integrity. MAPs also project lateral extensions that bridge between adjacent microtubules as well as between neurofilaments, microfilaments and membranous organelles. Although one or more MAP proteins are found in virtually all eukaryotic cell types, expression of tau, MAP2, MAP1A, and MAP1B is primarily neuronal.

The study of sporadic and familial neurodegenerative diseases over the past decade has led to the realization that many of these disorders are characterized by distinct hallmark brain lesions that have in common the formation of filamentous deposits of abnormal brain proteins. Thus, a group of heterogenous dementias and movement disorders

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Table 1. Diseases with tau-based neurofibrillary pathology [for details see ref. 1].

Alzheimer's disease
Amyotrophic lateral sclerosis/parkinsonism-dementia complex[a]
Argyrophilic grain dementia[a]
Corticobasal degeneration[a]
Creutzfeldt-Jakob disease
Dementia pugilistica[a]
Diffuse neurofibrillary tangles with calcification[a]
Down's syndrome
Frontotemporal dementia with parkinsonism linked to chromosome 17[a]
Gerstmann-Sträussler-Scheinker disease
Hallervorden-Spatz disease
Myotonic dystrophy
Niemann-Pick disease, type C
Non-Guamanian motor neuron disease with neurofibrillary tangles
Pick's disease[a]
Postencephalitic parkinsonism
Prion protein cerebral amyloid angiopathy
Progressive subcortical gliosis[a]
Progressive supranuclear palsy[a]
Subacute sclerosing panencephalitis
Tangle-only dementia[a]

[a] Diseases in which tau-positive neurofibrillary pathology is the most prominent neuropathological feature.

that are characterized neuropathologically by prominent intracellular accumulations of abnormal filaments formed by MAP tau appears to share common mechanisms of disease. They are collectively known as neurodegenerative tauopathies (table 1) [1].

Despite an ever-increasing body of data on many changes which tau undergoes in disease, its role regarding the fundamental disease process is still unclear. Moreover, the function of tau during normal brain development remains unsolved, which complicates an understanding of tau in the disease process.

In this article, the role of tau during normal brain development and normal regulation of its function is reviewed. In the second part, malfunctions of tau as they may be relevant for the development of diseases are discussed. At the end, experimental approaches to untangle open questions will be presented.

Tau structure

The tau proteins were originally isolated as a class of low-molecular-weight proteins, which co-purified with brain tubulin and promoted tubulin assembly into microtubules [2, 3]. Tau proteins are abundant in the central and peripheral nervous system, where they are expressed predominantly in neurons and are enriched in axons [4–6]. Subsequent molecular characterizations have shown that human tau proteins are encoded by a single gene located on chromosome 17q21. By alternative mRNA splicing of exons 2, 3, and 10, six tau isoforms are produced in the central nervous system (CNS) resulting in six different polypeptide chains with apparent molecular weights between 50 and 70 kDa (fig. 1). They differ by the presence or absence of one or two short inserts in the aminoterminal half (0N, 1N, and 2N, respectively), and have either three or four microtubule-binding repeat motifs in the carboxyterminal half (3R and 4R-tau). In fetal brain, only the shortest tau isoform (3R-tau) is expressed, whereas all isoforms are present in adult brain [1]. There is also a high-molecular-weight isoform (120 kDa), which is only expressed in neurons from the peripheral nervous system and which contains an additional exon in the aminoterminal half [7, 8]. The structure of the tau isoforms has been extensively reviewed in the past [7, 9, 10]. A striking feature of the primary structure of tau is the presence of three or four imperfectly repeated stretches of 31 and 32 residues in the carboxyterminal half, which constitute the core of the microtubule-interacting unit (fig. 2) [11]. The acidic aminoterminal region projects from the micro-

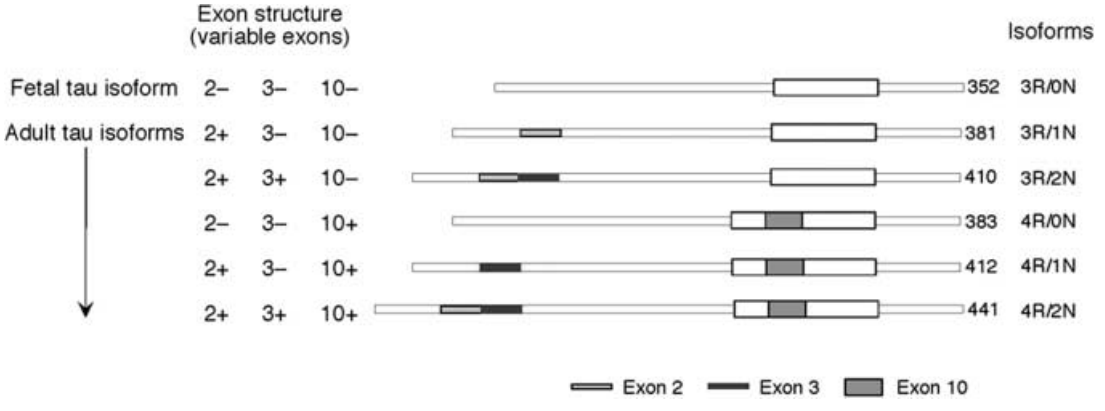

Figure 1. Schematic representation of the human tau isoforms. Fetal and adult isoforms of tau and adult-specific exons are shown. The microtubule-binding domain in the carboxyterminal half of the protein is indicated by the open box. Note that one of the exons (exon 10) codes for an additional repeat and that the expression of exon 3 requires the presence of exon 2. The high-molecular-weight species of tau expressed in peripheral nerves is not shown. This isoform contains an additional exon (4A) [8].

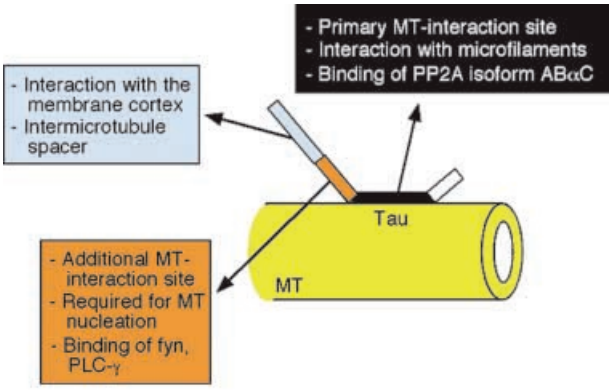

Figure 2. Functional organization of tau protein. Activities and interactions that have been mapped to specific tau regions are indicated. The repeat region is indicated in black, the proline-rich region in orange, and the aminoterminal projection domain in blue (MT, microtubule).

tubule surface when tau is bound to microtubules and may interact with other cytoskeletal elements and plasma membrane components [12, 13]. The middle region of tau, which is rich in proline (proline-rich region), contributes an additional microtubule interaction site and contains target sites for several kinases [14, 15].

Tau functions

During the past several years, different types of experimental approaches have been employed to obtain insight into functions of tau. Historically, tau has been described as a MAP with the role to promote microtubule stability and assembly. However, evidence is increasing for functions of tau beyond its ability to modulate microtubule dynamics. Here we will describe several functions attributed to tau to date.

Tau and microtubules

Tau was initially identified as a protein that bound to microtubules and stimulated microtubule assembly in cell-free reactions [2, 3]. These and other studies have determined that tau is capable of promoting microtubule nucleation, growth, and bundling [14, 15], as well as greatly reducing the dynamic instability of microtubules [16]. In addition, experiments with cultured rat fibroblasts showed that microinjection of tau promotes net microtubule accumulation and enhances microtubule stability [17], and that transfection and subsequent expression of tau leads to microtubule bundle formation and stabilization [18–20]. The repeat region of tau is the basic microtubule-interacting unit; however, sequences flanking the repeats considerably increase binding of tau to microtubules and are required for efficient de novo microtubule assembly with the centrally located proline-rich region being of particular importance (fig. 2) [14, 21, 22]. Phosphorylation of tau may be an important factor in regulating the interaction of tau with microtubules. Tau isolated from brain is phosphorylated at multiple sites and many kinases are capable of phosphorylating tau in vitro (fig. 3). Some phosphorylation events change the conformational state of tau [23], lead to decreased microtubule binding [24], lower its activity to promote microtubule assembly [25], and increase the dynamic instability of microtubules [15, 16]. Interestingly, phosphorylation of some individual residues, e.g., serine 262, which is located within the repeat domain, completely abolishes binding of tau to microtubules [26]. Phosphorylation of sites within the proline-rich region reduces the capacity of tau to promote de novo nucleation of microtubules in cell-free assembly reactions [27]. This suggests that the role of tau in temporally and spatially regulating neuronal microtubule assembly is modulated by its phosphorylation state.

Tau and neuronal polarity

Studies using cell culture models indicate that a fundamental role of tau is in neurite outgrowth and stabilization. Overexpression of tau in Sf9 insect cells results in the normally round cells sprouting long processes that resemble an axonal shape and are densely packed with microtubules [28] with a microtubule organization similar to axons [29]. In nerve growth factor (NGF)-treated PC12 cells, the induction of tau expression has been shown to correlate with an increase in microtubule mass, stability and process formation [30, 31]. Direct evidence supporting a role for tau in regulating axon formation and microtubule stability came from the analysis of the phenotype of cultured neurons in which the expression of tau protein was suppressed by means of treatment with antisense oligonucleotides. The first of these studies was performed in cerebellar macroneurons [32], a cell system in which the expression and subcellular distribution of tau is correlated with the morphological development of an axon [33]. Treated with tau antisense oligonucleotides, these macroneurons failed to extend axon-like processes while still being capable of extending short undifferentiated neurites (e.g., minor processes) [33]. In contrast, acute inactivation of tau by antibody microinjection had no effect on axonal elongation or the dynamics of microtubules in growing axons of cultured sympathetic neurons [34].

Given the conflicting results on the role of tau expression for axonal development in cultured neurons, data on the behavior of mice lacking tau are interesting. First evidence that tau was not essential for proper axonal formation came from analysis of the phenotype of tau knockout mice [35]. These mice developed normally, survived well, and did not develop major phenotypic changes. The nervous system of the tau-deficient mice was normal immunohis-

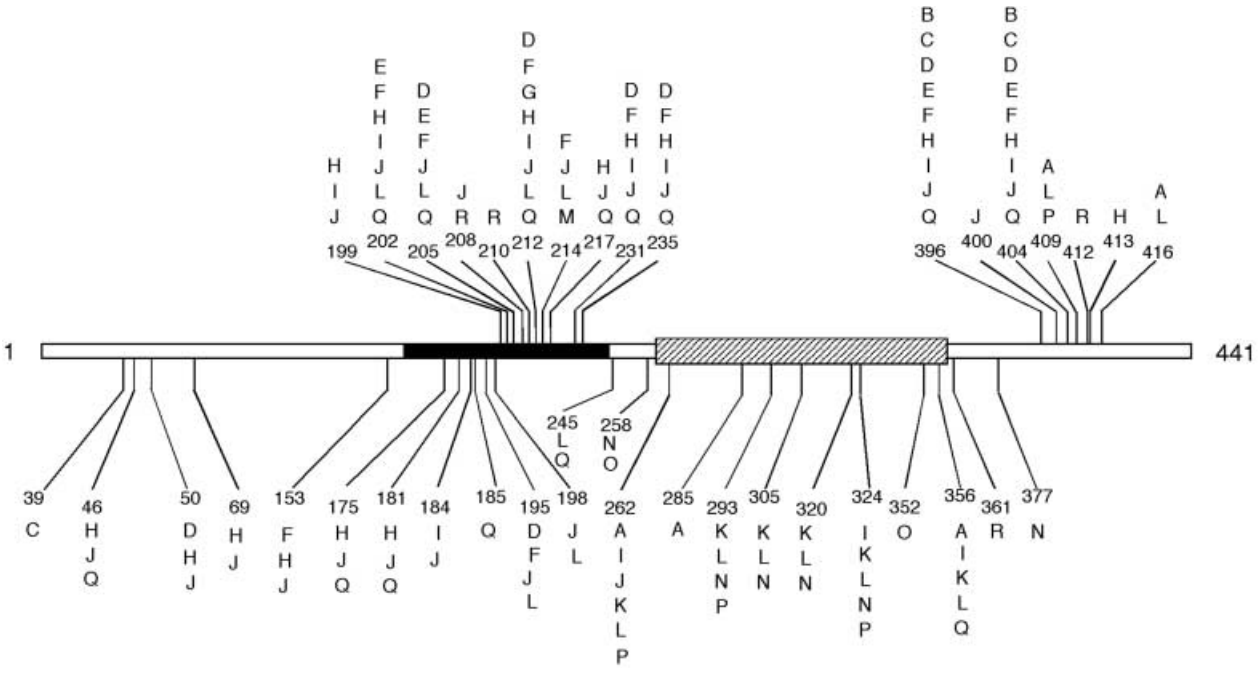

Figure 3. Sites of tau phosphorylation in vitro. The repeat region is hatched. Note that most of the phosphorylation sites are clustered in the proline-rich region (black box) and a carboxyterminal region flanking the repeats. In vitro phosphorylation sites for Ca^{2+}-calmodulin-dependent protein kinase II (A), casein kinase I (B), casein kinase II (C), cdc2 protein kinase (D), cyclin-dependent kinase 2 (E), cyclin-dependent kinase 5 (F), dual-specificity tyrosine phosphorylated and regulated kinase (G), mitogen-activated protein kinase (H), glycogen synthase kinase 3α (I), glycogen synthase kinase 3β (J), microtubule-affinity-regulating kinase (K), protein kinase A (L), protein kinase B/Akt (M), protein kinase C (N), PKN (O), 35/41-kDa protein kinase (P), stress-activated protein kinases (Q), tau tubulin kinase (R) are shown [for details see http://www.lille.inserm.fr/u422/TauPhosphoSeq.htm and ref. 54].

tologically and axonal elongation was not affected in cultured hippocampal neurons obtained from these animals. Only in some small-caliber axons (cerebellar parallel fibers) were microtubule density and stability decreased [35]. Recently, another tau knockout mouse was developed, which also appears phenotypically normal and is able to reproduce [36]. In contrast to the previously reported knockout mouse [35], primary cultures of hippocampal neurons from these mice show a significant delay in their axonal and dendritic extension [36]. Expression of human tau proteins in tau knockout mice rescues this delay in axonal sprouting, restores normal axonal growth and clearly shows that the phenotype is due to the absence of tau protein [36]. The data could suggest that there is significant plasticity and redundancy among the remaining MAPs to hide a required function of tau during normal development in the whole animal. In fact, an increased expression of another MAP, MAP1A, has been found in the first described tau knockout mouse [35]. This hypothesis has now been revisited by disrupting the genes for one of the two other prominent MAPs, MAP1B. Mice lacking both axonal MAPs, tau and MAP1B, died by 4 weeks after birth and the phenotypic changes were more severe than those observed in the individual knockout animals [37]. Cultured cerebellar neurons from these double-knockout animals exhibit suppressed axonal elongation. Additionally, a slight reduction in neuronal migration was observed in older layered structures of the brain (e.g., the hippocampus). Recent studies also show that in hippocampal neurons, during the transition from the minor process stage to the axonal stage, both MAP1B and tau are required [38]. Thus, this suggests that this pair of axonal MAPs function synergistically [37, 38]. Neurite outgrowth can be considered a specialized form of cell motility, and tau clearly plays a role in this process. Growing axons are very dynamic structures and the stability of the microtubules varies along the length of the axon. The distal end of the axon is more sensitive to microtubule depolymerizing drugs and the tubulin turnover is more rapid than in the proximal end. Assuming a role of tau as a microtubule-stabilizing protein, tau was hypothesized to be more bound to microtubules at the more stable, proximal end and less bound at the more dynamic, distal end. When this was tested in cultured rat sympathetic neurons, Black et al. [39] found the exact opposite. That is, tau was most associated with microtubules at the distal end of the axon close to the growth cone. A similar tau distribution was observed in cultured rat hippocampal neurons [40]. Since in the distal regions of growing axons, the microtubules are most dynamic, these data strongly suggest that tau in the growing axon has functions other than increasing microtubule stability.

In addition to concentration changes in tau protein in the developing neuron, the phosphorylation state of tau was also found to differ along the length of the growing axon [41]. Using fetal hippocampal cells, tau close to growth cones was found to be dephosphorylated at a particular site (Tau-1 site) compared to the somatodendritic compartment. A phosphorylation gradient was evident, with a gradual change from phosphorylated to dephosphorylated tau from the soma, through the axon, to the growth cone. These studies again suggest that the function of tau in the growing axon extends beyond its classical microtubule-stabilizing function, because in most cases, dephosphorylation of tau increases its affinity for microtubules.

Tau as a linker protein and its role in signal transduction

Evidence is increasing that tau interacts either directly or indirectly with the actin cytoskeleton and thereby plays a role in regulating cell shape, motility, and microtubule-plasma membrane interactions. Cells lacking the major actin-binding protein, ABP-280 (filamin), exhibit extensive membrane blebbing. Microinjections of tau or MAP2 prevented bleb formation and restored a normal phenotype [42]. Suppression of tau expression in cultured neurons using antisense oligonucleotides results in a significant reduction in growth cone area and fillopodia number concomitant with considerable changes in the phalloidin staining of actin filaments [43]. In addition, localization of tau to the distal axon requires intact microfilaments [40]. Although these data indicate that tau may interact with actin directly, tau more likely associates with the actin cytoskeleton through other proteins, as in vitro MAP2C efficiently promotes actin gelation, but tau does not [42]. Additionally, tau does not show a direct co-localization with the microfilament network, indicating the presence of binding partners, which could localize tau to specific positions of the actin cytoskeleton [40].

Tau has also been shown to interact with components of the neural plasma membrane cortex through its aminoterminal non-microtubule-binding domain, which protrudes from the microtubule surface when tau interacts with microtubules (fig. 2) [12]. This suggests a role for tau as a linker protein between microtubules and the membrane skeleton in the distal axon, e.g., at a site where the translation of the dynamic and transient movement of actin filaments into the more stable orientation of the microtubule arrays occurs. In vitro, tau interacts with spectrin [44], which may provide another link to the membrane skeleton. More recently, binding between the proline-rich sequence in the N-terminal part of tau proteins and the SH3 domains of src family non-receptor tyrosine kinases, such as fyn, was observed [45]. Moreover, the authors described the co-localization of tau and fyn just beneath the plasma membrane, and an association between the tau-fyn complexes and the actin cytoskeleton. These data favor a role for tau proteins in the src family tyrosine kinase signaling pathway that may modify the cell shape by acting on the submembranous actin cytoskeleton [45].

Co-immunoprecipitation of active phospholipase C-γ (PLC-γ) with tau in a human neuroblastoma cell line suggests a role for tau in the PLC-γ signaling pathway [46, 47]. Tau also acts as a phosphatidylinositol bisphosphate (PIP2)-binding protein that serves as a precursor for diacylglycerol and inositol trisphosphate in signal transduction cascades and regulates the activities of several actin-binding proteins that influence the organization of the actin cytoskeleton [48]. In addition, specific interactions of tau with the dominant protein phosphatase 2A isoform in the brain, ABαC [49], and tau-dependent targeting of protein phosphatase 1 to microtubules [50] have been described. These data may place tau in the context of signaling mechanisms involved in the development and maintenance of neuronal polarity linking these activities to the structural framework of the cytoskeleton.

Taken together, the functional data suggest that tau serves different functions which may be independently regulated by phosphorylation. Phosphorylation, in combination with the type of isoform, can modulate the properties of tau protein. In turn, tau proteins provide the microtubule with its own identity and physical characteristics (rigidity, length, stability, and interactive capacity with other organelles). Therefore, by regulating microtubule assembly, tau proteins may have a role in modulating the functional organization of the neuron, and particularly in axonal morphology, growth, and polarity.

Tau malfunctions

Tau was first implicated as a protein involved in the pathogenesis of Alzheimer's disease (AD) when it was discovered to be a major component of the neurofibrillary tangle originally described by Alois Alzheimer [51, 52]. AD, the most frequent cause of senile dementia, is characterized by a well-defined neuropathological profile which includes extracellular β-amyloid-containing senile plaques (consisting mainly of aggregated Aβ peptide derived by proteolysis of the amyloid precursor protein, APP), intracellular neurofibrillary tangles (NFTs), reduced synaptic density, and neuronal loss in selected brain areas. NFTs are mainly composed of hyperphosphorylated tau. The number of NFTs is, in contrast to the distribution of the senile plaques, closely correlated with the degree of dementia. NFTs develop in a characteristic sequence of events during AD. The initial changes are seen in the entorhinal cortex from which the destructive process then spreads into the hippocampal formation and eventually the isocortex [53]. NFTs are, in the absence of amyloid plaques, also abundant in other neurodegenerative dis-

eases including Pick's disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, and frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17).

In nerve cells affected in these tauopathies, tau is abnormally phosphorylated and relocalized from axonal to somatodendritic compartments where it accumulates in pretangle, filamentous aggregates (PHFs or paired helical filaments in AD) that eventually assemble into NFTs [54, 55]. In contrast to AD, where hyperphosphorylated tau forms filaments only in neurons, numerous tau-filament-containing glial cells are present in a variety of tauopathies including progressive supranuclear palsy and corticobasal degeneration. Not known at present is whether the glial pathology affects neuronal degeneration or is required for progression of disease [56].

Tau in PHFs is extensively modified posttranslationally by phosphorylation, glycation, racemization, and ubiquitination [54]. Increased phosphorylation of tau (hyperphosphorylation) appears to be an early modification in the pathological cascade, since immunohistochemical data demonstrated the occurrence of tau-specific phosphoepitopes early during the disease process [57]. Tau isolated from PHFs has a stoichiometry of phosphorylation of about 6–8 mol phosphate/mol tau, which is significantly higher than in tau isolated from control brains (1.9 mol phosphate/mol tau) [58]. While few if any phosphorylation sites appear to be unique to PHF-tau, the proportion of tau phosphorylation at any given site is significantly higher in PHF-tau than in biopsy-derived normal tau protein [59]. The hyperphosphorylation of tau proteins associated with AD may be related to either an increase in kinase activity or a decrease in phosphatase activity [60]. Thus, hyperphosphorylation of tau could represent a critical event leading to mislocalization and abnormal aggregation of tau proteins in affected neurons. PHF-tau from AD patients is almost incapable of promoting microtubule assembly in vitro and this activity can be restored after dephosphorylation of tau protein [61–63].

Tau aggregation in AD was known for more than a decade before a genetic disease finally demonstrated that tau pathology can be primary in dementia. The recent discovery of mutations in the tau gene in FTDP-17 has established that dysfunction of tau in itself can cause neurodegeneration and lead to dementia [64–66]. FTDP-17 is associated with both exonic and intronic mutations of the tau gene. Intronic and some exonic mutations affect the alternative splicing of exon 10 and, consequently, alter the relative proportion of 4R-tau and 3R-tau isoforms [67–70]. The other exonic mutations impair the ability of tau to bind microtubules and to promote microtubule assembly, and some of these mutations also promote the assembly of tau into filaments [71–78].

Despite the identification of mutations in FTDP-17, many questions about the role of tau in neurodegenerative tauopathies remain unsolved. Uncertain is how tau dysfunction leads to the formation of tau inclusions, what role hyperphosphorylation plays in the aggregation of tau, and how the formation of inclusions relates to other pathogenic events, such as neuronal loss. Hence, in the following section, we discuss a mechanistic framework for the involvement of tau during the disease process.

Tau: loss of function?

Intraneuronal filamentous inclusions composed of abnormally hyperphosphorylated tau are a feature of several neurodegenerative diseases known as tauopathies. Tau hyperphosphorylation or mutations in the tau gene have been proposed to cause a functional loss of tau for interaction with microtubules and render tau inactive to promote microtubule stability. As a result, axonal microtubules would be destabilized and eventually depolymerize. Such a sequence of events is likely to severely affect fast axonal transport, which occurs mainly on microtubule tracks, and would cause neurons to degenerate (fig. 4).

A critical assumption in this view is that neurons depend on tau for stabilization of their axonal microtubule array. However, the pathological relevance of such a 'loss of function' scenario toward microtubules during the disease process appears unlikely based on the experiments where tau or the tau gene had been inactivated in cells and animals. As discussed before, tau knockout mice are quite normal and lack major cytoskeletal abnormalities, arguing against an essential role of tau for the maintenance of axonal structure [35]. In agreement, immunodepletion of axonal tau in cultured neurons does not affect axon growth or the distribution and stability of axonal microtubules [34]. Furthermore, analysis of the distribution of tau in cultured neurons has shown that tau is particularly enriched in the distal axon at a position where axonal microtubules are known to be most dynamic and least stable [39–41]. This is opposite to what would be expected from the distribution of a microtubule-stabilizing agent.

Tau: toxic gain of function?

Another point of view is that not the loss of function of tau but the formation of large intraneuronal aggregates of tau acts either as a physical barrier to axonal transport and other essential neuronal functions or has direct toxic effects on cells. In such a scenario, tau aggregation may represent a 'toxic gain of function' rather than a loss of function during the disease (fig. 4). In support of this view, a five- to tenfold overexpression of tau in transgenic animals leads to the formation of tau-containing intraneuronal inclusions in cortical, brain stem, and spinal cord neurons where the presence of aggregates is associated with axonal degeneration [79]. The formation of inclu-

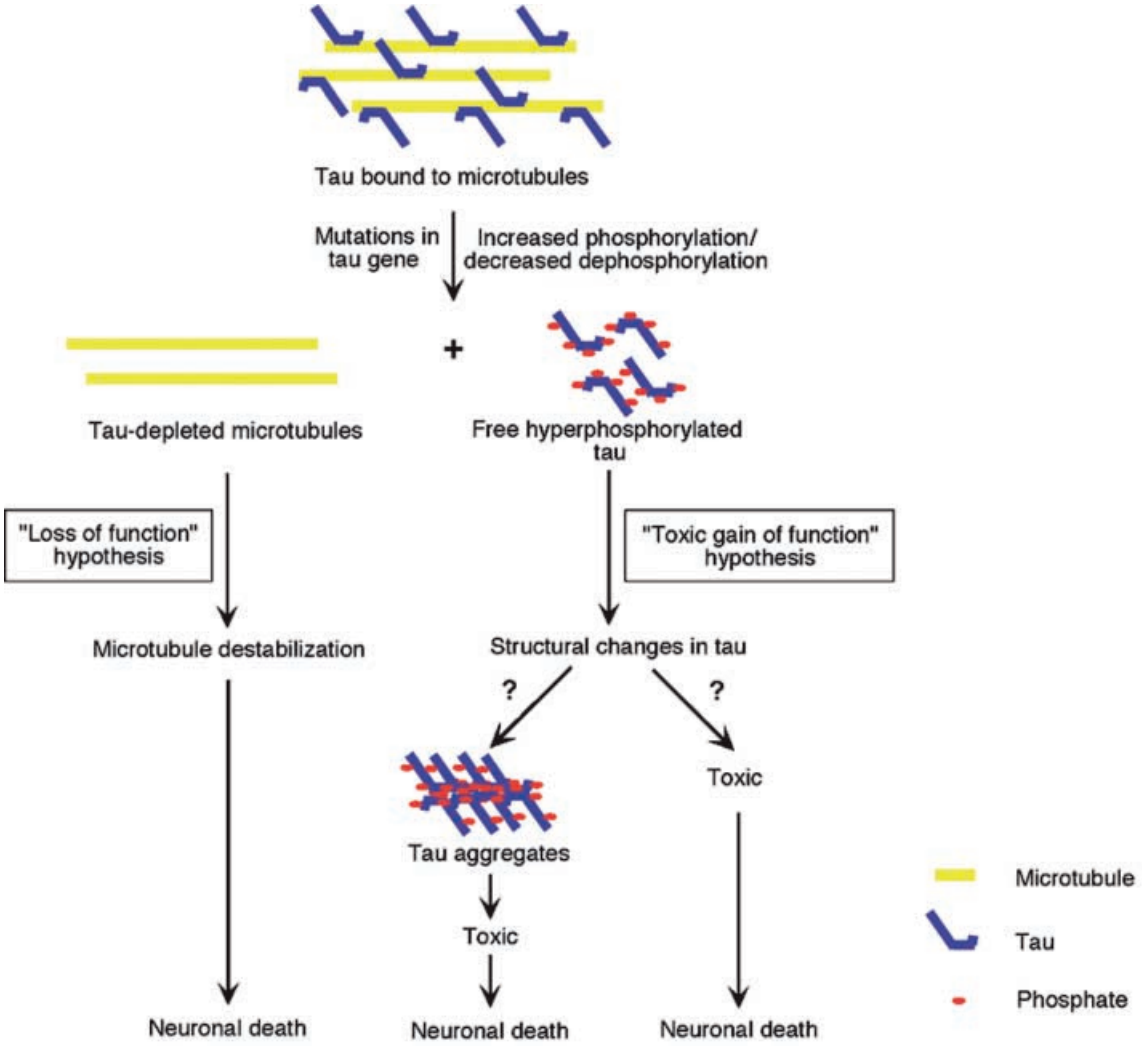

Figure 4. Tau: 'loss of function' or 'toxic gain of function' model. Tau binding to microtubules is disrupted by changes in phosphorylation or by mutations in the tau gene. Loss of tau binding may result in loss of microtubule function. On the other hand, decreased tau binding to microtubules might result in increased hyperphosphorylated free tau. This may cause aggregation of tau into neurofibrillary tangles and neuronal death. Another possibility is that hyperphosphorylated tau itself is toxic to neurons as a result of structural changes induced by phosphorylation.

sions appears to depend on the concentration of tau, because the expression of low levels of tau (less than twofold compared with the endogenous tau concentration) does not cause formation of tau aggregates in transgenic mice [80, 81]. Furthermore, transgenic mice carrying FTDP-17 tau mutations exhibit features of human tauopathies, including the development of NFTs and axonal degeneration [82, 83].

Hyperphosphorylation of tau may have a more indirect role during the pathology by increasing the amount of free tau protein for the subsequent formation of tau aggregates in the affected neurons. In aggrement with this view, hyperphosphorylated tau from AD brain cytosol has recently been shown to assemble into tangles of PHFs and straight filaments and phosphorylation is essential for its self assembly, which is abolished by dephosphorylation [84]. Recently, Jackson et al. [85] observed that 4R-human tau overexpression, in combination with phosphorylation by the *Drosophila* glycogen synthase kinase-3β (GSK-3β) homolog Shaggy exacerbated tau-induced neurodegeneration, resulting in the formation of NFT-like filamentous tau aggregates. This study shows a causal relationship between tau hyperphosphorylation and neurofibrillary pathology in vivo. However, in contrast to this view, other evidence indicates that tau phosphorylation does not increase or even reduces filament formation in vitro [86, 87], which would argue against a role of phosphorylation of tau to facilitate filament formation. In agreement, when hyperphosphorylation of tau was simulated by site-directed mutagenesis, tau protein exhibits reduced filament formation compared to wild-type tau [88].

One should note, however, that in vitro filament assays depend on the addition of polyanions (e.g., heparin or RNA) or anionic lipid-derived substances (e.g., arachidonic acid) to increase tau aggregation [89, 90]. Recently, in vitro formation of filamentous aggregates of phosphorylated tau protein in human neuroblastoma cells was also shown to require okadaic acid (a phosphatase inhibitor) to increase the level of phosphorylated tau, and hydroxynonenal, a product of oxidative stress that selectively adducts and modifies phosphorylated tau [91]. However, whether both tau phosphorylation and oxidative modification contribute to tau filament formation requires further verification. In contrast to a previous study [85], double transgenic mice, which overexpressed GSK-3β (known to phosphorylate tau and to decrease the tau-microtubule interaction) and 4R-tau show decreased axonal pathology compared to mice overexpressing 4R-tau alone [92]. This would suggest that an increase in tau protein, and not hyperphosphorylation, is responsible for the neuropathological phenotype in these tau transgenic mice. However, although more hyperphosphorylated tau was available, PHF formation was not observed [92]. Accordingly, overexpression of tau alone in *Drosophila* induces neurodegeneration without the formation of NFTs [93], which argues that, in the case of tau, neurotoxicity instead depends on protein alterations that occur before the formation of aggregates. This observation, in conjunction with the neurodegeneration seen after overexpression of wild-type 4R-human tau alone in *Drosophila* [85], suggests that neurotoxicity is due to abnormal phosphorylation of tau per se, rather than to NFT formation. Thus, the data may suggest that, in certain conditions, soluble (i.e., monomeric or oligomeric) tau protein exerts neurotoxic activity in the brain.

As a further alternative, the hyperphosphorylation state of tau itself may represent a toxic insult for the neuron, independent of the formation of filaments. Interestingly, pseudohyperphosphorylated tau that simulates disease-like permanent, high-stoichiometric tau phosphorylation and mimics structural and functional aspects of hyperphosphorylated tau [88, 94, 95] exerted a neurotoxic effect in neural cells, which was associated with induction of apoptosis [Fath, Eidenmüller, Brandt; J. Neurosci., in press]. These data provide evidence for a 'neurotoxic gain of function' of soluble (i.e., monomeric or oligomeric) tau as a result of structural changes that are induced by cumulative, high-stoichiometric tau phosphorylation, which was independent of tau aggregation. If tau hyperphosphorylation itself represents the toxic insult, the formation of tau aggregates could even represent a rescue mechanism to reduce the amount of free tau available in the cell (fig. 4).

In addition to binding to microtubules, tau has been shown to bind to neural plasma membrane components through its aminoterminal non-microtubule-binding domain, and this interaction may be important for axon-specific localization of tau [12, 40]. An increase in tau phosphorylation and simulation of tau hyperphosphorylation completely abolish the interaction of tau with the membrane cortex [94], and this may cause mislocalization of tau thus contributing to a loss of tau from axonal microtubules and increasing tau accumulation in the cell body.

Tau: linkage to amyloid plaques?

The fact that mutations in tau give rise to tau-inclusion tangles but not plaques and yet mutations in APP give rise to both plaques and tangles [96, 97] suggests that amyloid pathology occurs upstream of tau pathology. The mutations in tau have, however, emphasized that this tau-related pathology can be sufficient to cause dementia.

Although these two lesions are present in the same brain areas, a mechanistic link between them has yet to be established. Deposition of fibrillar Aβ was earlier reported to induce phosphorylation of tau followed by progressive degeneration of neuronal processes [98, 99]. A recent report showed that cultured tau-depleted hippocampal neurons (obtained from tau knockout mice) do not degenerate in the presence of fibrillar Aβ [100]. In addition, analysis of the composition of the cytoskeleton of these tau-depleted neurons suggested that the formation of more dynamic microtubules might confer resistance to Aβ-mediated neurodegeneration. Overexpression of tau in cultured fibroblasts [101] and neural cells [102] was reported to inhibit the transport of cell components and leave microtubules intact. Tau inhibited kinesin-dependent transport of peroxisomes, neurofilaments, and Golgi-derived vesicles into neurites. This may make cells vulnerable to oxidative stress and lead to degeneration. In particular, tau inhibited transport of APP into axons and dendrites, causing APP accumulation in the cell body [102], which would allow an enhanced production of toxic Aβ peptides [103]. Work described by Lewis et al. [104] and Götz et al. [105] provides convincing evidence for a causal connection between the NFTs and plaques. Both groups independently demonstrated in transgenic mice that amyloid-β deposits influence the formation of tau tangles in brain areas known to be affected in AD. A transgenic mouse overexpressing both mutant APP and mutant tau has both plaques and tangles [104]. This in itself is not surprising given that the mice were derived from parental lines that exhibited either plaque pathology (due to overexpression of mutant APP) or extensive tangle pathology (due to overexpression of mutant tau). However, of interest is that the double mutant has more tangles than tau mutant mice alone and tangles appear in brain areas that are unaffected in single-mutant tau transgenic mice. In a parallel experiment, another group showed that injection of fibrillar Aβ into the hippocampus of mutant tau transgenic mice exaggerated tangle pathology in the amygdala, one of the regions affected in AD. Intriguingly, tau tangles did not de-

velop in the hippocampus, the site where fibrillar Aβ was injected, but rather appeared in the amygdala, a site to which hippocampal neurons project [105].
Further evidence shows that the plaque itself does not induce tau pathology because the typical AD plaques surrounded by dystrophic neurons filled with aggregated and phosphorylated tau were not induced [104]. Nor does a failure of normal APP function seem to be involved in exacerbating the neurofibrillary pathology, because in the injection model, the only perturbation is an addition of fibrillar Aβ, endogenous APP being unaffected. Whatever the mechanism, these models clearly demonstrate that exposure to fibrillar Aβ alone is not sufficient to induce tangle formation because tangles were not induced when fibrillar Aβ was injected into the hippocampus of mice overexpressing wild-type tau [105].
Hence, developing cellular and animal models to verify and analyze the role of tau protein in neurodegeneration and the functional interaction between tau and other disease factors will be very important. In addition, given a potentially critical role of tau phosphorylation during the disease process, determining how PHF-specific phosphorylation events affect cellular interactions and functions of tau will also be important. In the last part of this review, we will discuss some experimental approaches to elucidate the functions and malfunctions of tau.

New approaches to study the functional role of tau

Cellular model approaches

To analyze the intracellular mechanisms which contribute to disease-related malfunctions of tau, appropriate cellular and animal models must be developed. Most of the cytoskeletal mechanisms that occur during neuronal development were analyzed in simple culture models, where neurons were dissociated and brought into culture. The basic processes that have been observed in these 'naked' neurons can be anticipated to remain the same in a more complex environment. Cellular models have been an important tool to analyze mechanisms which are involved in producing a hyperphosphorylation state of tau, which may represent an early state in tau pathology. Overexpression of tau in cultured cells does not lead to PHF formation although changes in tau phosphorylation and localization have been found. Only recently, results suggested that both phosphorylation and oxidative modification contribute to tau filament formation in a human neuroblastoma cell line [91]. Confirming these results in other systems will be important.
While very useful for screening purposes, neural cell lines and dissociated neuronal cultures exhibit inherent limitations due to the artificial environment in which they develop. For example, processes in the membrane cortex especially can be expected to be affected by the presence of cellular contacts. In fact, tau, which clearly shows an enrichment in axons in situ, is ubiquitously present in dissociated neuronal cultures unless special extraction protocols to reveal association of tau with microtubules are employed [4, 40]. Thus, to confirm in vitro results and to include the potential role of cell-cell interactions, conditions must be developed where neurons can be analyzed in a more complex environment.

Approaches using transgenic mice

Advances in genetics and transgenic approaches have a continuous impact on our understanding of the role of tau and other proteins in neuronal functions and pathology, especially as aspects of the histopathology and neurodegeneration can be reproduced in animal models. As discussed before, mice deficient in the expression of tau show only minor phenotypic changes [35], although in cultured neurons suppression of tau expression inhibits the formation of an axon [32]. Most probably this is due to the fact that many cytoskeletal mechanisms show some functional redundancy. Using transgenic animals, evidence has been provided that a causal connection exists between the tau and amyloid pathologies.
Furthermore, mutations in tau have emphasized that tau-related pathology could be sufficient to cause dementia. The currently available and newly established FTDP-17 mutant transgenic models [106] will be of help to determine which phosphoepitopes of tau are important for filament formation in vivo, which cells are susceptible to filament formation, which subcellular compartments are capable of making tau filaments, how filaments interfere with neuronal functions including axonal transport, and whether glial cells with tau filaments can influence neuronal functions. In the long run, they will provide insight into the physiological role of tau, and, eventually, the components of the pathocascade in AD and frontotemporal dementia will be identified. Finally, these mice will be available for drug testing aimed at halting or preventing AD and related tauopathies. On the other hand, due to the apparent redundancy of cytoskeletal mechanisms, the phenotypes of mice deficient in individual cytoskeletal components appear to be difficult to interpret and may require multiple knockouts in different combinations to allow mechanistic interpretations. However, production of transgenic rodents requires much time. Thus, at least in the short run, other methods may be more effective to understand the role of tau and other disease-relevant proteins.

Organotypic slice culture and the simple animal model approach

A potential alternative approach is to disturb cellular functions by overexpressing or downregulating tau in an au-

thentic CNS environment (organotypic slice cultures) or simple animal models. Organotypic slice cultures of neural tissue (e.g., from rat or mice hippocampus) have been extensively used as a model system to study neuronal plasticity [107] and to investigate the regulation of tau phosphorylation [108]. Organotypic hippocampal cultures combine the accessibility and maintenance of in vitro culture systems while preserving intact the hippocampal synaptic circuitary and anatomy [109, 110]. To perturb cellular functions, effective methods to express exogenous proteins or antisense constructs need to be developed. Recombinant Semliki Forest virus and Sindbis virus may provide a useful system for efficient expression of exogenous proteins in neurons in hippocampal slices [111]. Expression of fluorescently tagged tau constructs may provide an elegant method to determine the role of tau and its modifications as well as their interactions with other factors involved in the disease process.

In addition, simple animal models, which allow a fast and efficient production of transgenic animals, could provide a useful system to functionally analyze tau proteins in a systemic context. An example is the targeted expression of human tau protein in neurons in the fruit fly *Drosophila melanogaster* that produces characteristic defects in the expressing neurons [112] and tauopathy [85, 93]. Simple animal models could also allow correlation of changes in the nervous system with behavioral defects as has been demonstrated with transgenic nematode (*Caenorhabditis elegans*) expressing mutated presenilin constructs involved in some familiar forms of AD [113, 114].

Taken together, increasing evidence suggests a complex interaction between tau and other factors involved in neurodegenerative tauopathies. Approaches using different cell culture and animal models promise to be of help to elucidate critical questions with respect to the role of tau modification, tau aggregation, and functional interactions of tau during the disease process. They may be of help to establish screening systems capable of testing drugs that interfere with tau malfunction during disease.

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