Tau-mediated neurodegeneration in Alzheimer's disease and related disorders

Carlo Ballatore§, Virginia M.-Y. Lee*‡ and John Q. Trojanowski*‡*

Abstract | Advances in our understanding of the mechanisms of tau-mediated neurodegeneration in Alzheimer's disease (AD) and related tauopathies, which are characterized by prominent CNS accumulations of fibrillar tau inclusions, are rapidly moving this previously underexplored disease pathway to centre stage for disease-modifying drug discovery efforts. However, controversies abound concerning whether or not the deleterious effects of tau pathologies result from toxic gains-of-function by pathological tau or from critical losses of normal tau function in the disease state. This Review summarizes the most recent advances in our knowledge of the mechanisms of tau-mediated neurodegeneration to forge an integrated concept of those tau-linked disease processes that drive the onset and progression of AD and related tauopathies.

Senile plaque
A site of Aβ accumulation and dystrophic neurites in the brains of mouse models and patients with Alzheimer's disease.

**Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania, 3600 Spruce Street, Philadelphia, Pennsylvania 19104-4283, USA.*
‡Institute on Aging, University of Pennsylvania, 3615 Chestnut Street, Philadelphia, Pennsylvania 19104-2676, USA.
§Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, Pennsylvania 19104-6323, USA.
Correspondence to J.Q.T.
e-mail: trojanow@mail.med.upenn.edu
doi:10.1038/nrn2194
Published online
8 August 2007

A growing body of evidence suggests that the generation of proteinaceous aggregates is a common pathological process in numerous neurodegenerative diseases. Indeed, not only is the defining characteristic of several neurodegenerative diseases the accumulation of proteinacious fibrillary substances (such as senile plaques (SPs) made of β-amyloid (Aβ), or neurofibrillary tangles (NFTs) made of tau), but significant circumstantial evidence also clearly implicates these aggregates in the onset and progression of most aging-related neurodegenerative disorders that manifest clinically with progressive cognitive and/or motor impairments. In the case of neurodegenerative tauopathies — a group of disorders that includes Alzheimer's disease (AD) and the frontotemporal dementias (FTDs) — NFTs consisting of aggregated straight or paired helical filaments (SFs and PHFs, respectively), twisted ribbons or other conformations[1] of aberrantly phosphorylated forms of the microtubule-associated protein (MAP) tau are the diagnostic hallmark lesions in the CNS. Although the precise role of these and other specific diagnostic lesions in the different stages of neurodegenerative disease pathology is not yet fully understood, it is increasingly evident that tau-mediated neurodegeneration may result from the combination of toxic gains-of-function acquired by the aggregates or their precursors and the detrimental effects that arise from the loss of the normal function(s) of tau in the disease state. Elucidating the exact roles of the different aggregates and their precursors in neurodegeneration is a challenging endeavor, but one that is likely to remain the focus of future research efforts to discover the mechanisms of disease pathology, as well as to develop better diagnostics and therapeutics.

Thus far, several lines of investigation have suggested different, and at times contradictory, cause-and-effect relationships between various pathological species of disease proteins and the aggregates that they form. These apparent contradictions may reflect the limitations inherent in each of the *in vitro* and *in vivo* model systems that are used to study specific disorders, including neurodegenerative tauopathies. For example, the realization that the neurotoxic species that contribute to disease onset and progression may be 'hidden' in one or more of the pre-aggregated/pre-fibrillar forms of the misfolded protein clearly complicates both experimental design and the unambiguous interpretation of results. Moreover, added complexity may come from the fact that, aside from its well-established role in promoting the stabilization of microtubules (MTs), tau may have additional functions as a result of its interactions with other structures and enzymes[1] (for example, with the plasma membrane[2,3], the actin cytoskeleton[4] and with src tyrosine kinases such as FYN[5] (see below)). Such poorly-defined interactions and functions of tau contribute to the difficulty of understanding how pathologically altered tau mediates neurodegeneration, and more studies

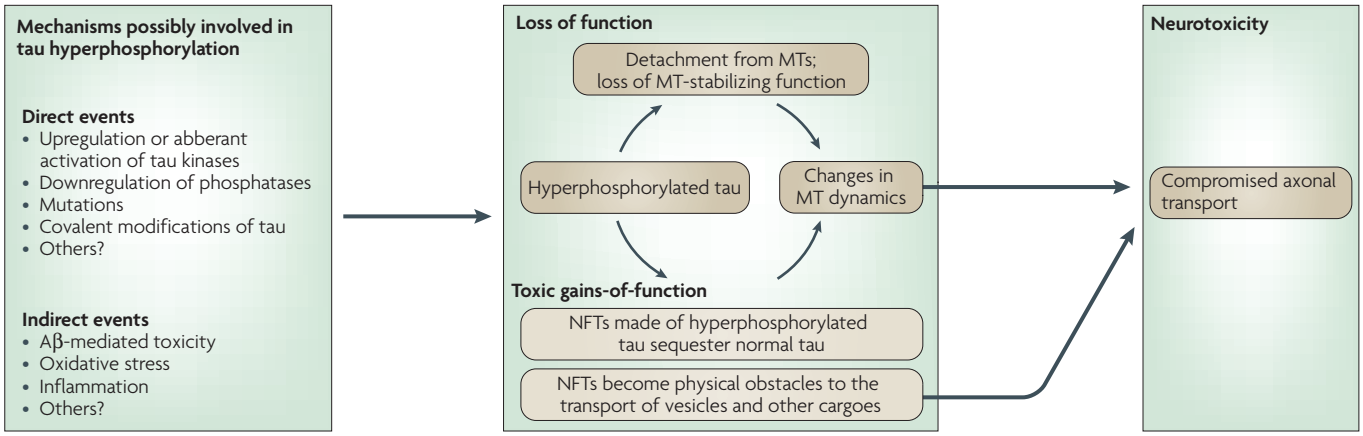

Figure 1 | **Direct and indirect pathological events that can contribute to tau-mediated neurodegeneration.** Pathological events that can contribute to tau-hyperphosphorylation and detachment from microtubules are shown in the box on the left. The middle box shows the mechanisms that underlie the loss of normal function and toxic gain-of-function of tau, which ultimately result in impaired axonal transport and lead to synaptic dysfunction and neurodegeneration (right hand box). Aβ, amyloid-β; MT, microtubule; NFT, neurofibrillary tangle.

are needed in order to elucidate these mechanisms. In addition, because disease onset and progression are dynamic processes that take place over time (often over several years), it is conceivable that processes such as the aggregation of altered tau may produce a range of different effects at various stages of the disease.

Given the complexities of this research, it is timely to critically analyse the progress made towards a mechanistic understanding of tau-mediated neurodegeneration, and to discuss the therapeutic strategies that target the most severe toxic consequences of tau pathologies. To that end, our goal is to summarize the current understanding of normal tau functions and the pathogenesis of tau aggregates in AD and related neurodegenerative tauopathies, as well as their significance to the onset and progression of these disorders (FIG. 1). We also provide an overview of the animal models of tau-mediated neurodegeneration (BOX 1; TABLE 1), and discuss tau-directed drug-discovery efforts (BOX 2; TABLE 2).

Box 1 | **Animal models of tau pathology**

The use of invertebrates and rodents in reproductions of human neurodegenerative diseases that affect higher cognitive functions over an extended period of time (decades), although faced with numerous challenges, has been undeniably instrumental in gaining insights into the mechanisms that underlie these diseases[64,75-77]. Indeed, although none of the available models is capable of recapitulating all the features of tangle pathology that are found in the human brain, various transgenic models (TABLE 1) are capable of reproducing different features of Alzheimer's disease (AD) in humans, and triple-transgenic mice can now reproduce multiple signature lesions such as plaques and NFTs in the same animal model. As such, these transgenic animal models comprise the most effective tool available to date for studying AD and related tauopathies. Some of the commonly used transgenic animal models are typically associated with the development of motor impairments, which can severely limit the use of these models in behavioural tests. However, recent lines of research[78] have led to the development of transgenic tauopathy models that have no overt signs of motor deficits. These particular models will certainly be invaluable for evaluating the effects of candidate drugs on cognitive decline.

Physiological functions of tau

The primary function of the MAP tau, which is particularly abundant in the axons of neurons, is to stabilize MTs. As summarized in FIG. 2, there are six major isoforms of tau expressed in the adult human brain, all of which are derived from a single gene by alternative splicing. From a structural stand-point, tau is characterized by the presence of a MT-binding domain, which is composed of repeats of a highly conserved tubulin-binding motif[6] and which comprises the carboxy-terminal (C-terminal) half of the protein, followed by a basic proline-rich region and an acidic amino-terminal (N-terminal) region, which is normally referred to as the 'projection domain'. The six tau isoforms differ from each other in the number of tubulin-binding repeats (either three or four, hence the isoforms are normally referred to as 3R and 4R tau isoforms, respectively) and in the presence or absence of either one or two 29 amino-acid-long inserts at the N-terminal portion of the protein, which is not instrumental for MT-binding[7]. Although the six isoforms appear to be broadly functionally similar, each is likely to have precise, and to some extent distinctive, physiological roles. The various isoforms appear to be differentially expressed during development, however, the 3R and 4R tau isoforms are expressed in a one-to-one ratio in most regions of the adult brain, and deviations from this ratio are characteristic of neurodegenerative FTD tauopathies[8].

Several lines of investigation substantiate a model whereby the tubulin-binding repeats bind to specific pockets in β-tubulin at the inner surface of the MTs, whereas the positively charged proline-rich regions are

Alternative splicing
The process by which introns are excised from RNA after transcription and the cut ends of the RNA are rejoined to form a continuous message. Alternative splicing allows the production of different messages from the same DNA molecule.

Table 1 | **Commonly used transgenic mouse models of tau pathology**

Gene	Mutation/construct	Promoter	Tau pathology	Refs
Mapt	4R/2N isoform	Thy1	Hyperphosphorylated PHFs	82
			Axonopathy without formation of neuronal NFTs	83
			Axonopathy containing neurofilament- and tau-immunoreactive spheroids, especially in the spinal cord	84
	Fetal tau (3R/0N isoform)	Prion protein promoter	NFTs in the brain at 18 months of age	56,85
	P301L	Prion protein promoter	Tangle pathology detectable at 2.5 months of age	60,86
	P301L	Thy1.2	Tangle pathology detectable at 3 months of age	87
	Inducible overexpression of P301L	Ca^{2+}–calmodulin-dependent kinase II	Tangle pathology detectable at 2.5 months of age	13
	Genomic tau	Endogenous	Tau-immunoreactive axonal swellings	88
	G272V	Prion protein promoter	Oligodendroglial fibrillary lesions	89
	P301S	Thy1.2	Tau pathology detectable at 5 months of age	90
	G272V P301L R406W	Thy1	Tau pathology detectable at 1.5 months of age. No motor impairment observed for up to 12 months after birth	91
	V337M	PDGF-β	Mutant tau induces neuronal degeneration, associated with the accumulation of RNA and phosphorylated tau	92
	R406W	Ca^{2+}–calmodulin-dependent kinase II	Hyperphosphorylated tau inclusions appear in the forebrain at 18 months of age. No motor abnormalities for up to 23 months after birth	93
	G272V P301S	Thy1.2	Hyperphosphorylated tau, tangles and PHFs. No motor impairment for up to 18 months after birth	78
	P301S	Prion protein promoter	This model recapitulates tauopathy, including early indications of degeneration, such as synapse loss and microglia activation	63
Apolipoprotein E (*Apoe*)	ApoE4	Multiple	Phosphorylated tau expression in the neocortex, the hippocampus and the amygdala	94
Cdk5r1	p25	Neuron-specific enolase	Phosphorylated tau expression in the cortex, the amygdala and the thalamus	95
VKαD11HuCκ and *VKαD11HuCγ*	Anti-NGF IgH/Igκ	Cytomegalovirus early region	Phosphorylated tau expression in the cortex and the hippocampus, with associated neuron loss	96
App, *Psen1*, and *Mapt*	PS1 (M146V), APP (Swe), tau (P301L)	Thy1.2	Both tau and Aβ pathology	97,98

All transgenic mice overexpressed their transgenic protein. *VKαD11HuCκ* and *VKαD11HuCγ* carried the light and heavy chain genes of the chimeric antibodies αD11, respectively. Aβ, amyloid-β; App, amyloid-b precursor protein; Ig, immunoglobulin; NFT, neurofibrillary tangle; NGF, nerve growth factor; PDGF-β, platelet-derived growth factor-β; PHF, paired helical filament; PS1, presenilin 1.

tightly bound to the negatively charged MT-surface, and the negatively charged projection domain branches away from the MT-surface, possibly owing to electrostatic repulsion[9,10]. Interestingly, it was found that the MT-binding domain of tau, and several MT-stabilizing drugs, including paclitaxel, epothilone and discodermolide, seem to share, either completely or in part, the same binding pockets in the β-tubulin[9,11]. Although the occupation of these pockets by tau, other MAPs or MT-stabilizing drugs is thought to be sufficient to maintain the tubulin conformations that promote the polymerized state, MAPs, unlike MT-stabilizing agents, may also contribute to MT-stabilization in other ways. It is believed that the β-tubulin pockets of adjacent protofilaments may be occupied by the different repeats of the same MT-binding domain of tau, thereby causing the crosslinking of three or four dimers[9]. In addition, interactions of the proline-rich region of tau with the surface of the MTs are likely to further contribute to MT stabilization.

Interestingly, although the primary function of the MT-binding domain of tau is the stabilization of MTs, various lines of investigation have indicated that it may also engage with other structures and enzymes, including RNA[12] and presenilin 1 (PS1)[13]. Similarly, numerous possible binding partners have been proposed for both the proline-rich and the projection domains (the SH3 domains of src-family tyrosine kinases such as FYN[5], and the plasma membrane[2,3], respectively). Although the importance of these specific interactions of tau with partner structures other than the MTs is not yet known in the context of tau-mediated neurodegeneration, collectively these findings support the notion that tau might be a rather promiscuous binder that is prone

Box 2 | **The development of therapeutics**

The need for therapies that are capable of modifying both amyloid-β (Aβ)- and tau-mediated neurodegeneration cannot be overemphasized. Although a relatively large number of Aβ-directed therapeutic approaches have been proposed, and although many of these are at various stages of clinical investigation[79], tau-directed therapies have been lagging behind. Nonetheless, in light of the fact that two of the most extensively studied cancer targets, namely kinases and MTs, are clearly involved in tau-mediated neurodegeneration, the search for therapeutics that are capable of modifying tau-pathology will be able to take advantage of past experience and, importantly, of the relatively large number of biologically active compounds that have already been developed. In addition, thanks to the recent development of various *in vitro* tau fibrillization assays, high-throughput screening (HTS) of compound libraries has been possible, and this has led to the identification of structures that are capable of functioning as imaging ligands to detect aggregates in living patients, inhibiting tau fibril formation and/or dissolving preformed fibrils[66–69,80] (TABLE 2). Although these HTS efforts are clearly part of rather early-stage drug-discovery programmes, the hits discovered thus far have promise as novel molecular tools to further investigate the pathophysiology of tau. However, in order to conduct thorough evaluations of the existing therapeutic agents, as well as of novel candidate compounds, in the context of neurodegenerative diseases, key pharmacokinetic issues such as drug uptake in the brain, which is notoriously hampered by the presence of the highly discriminating blood–brain barrier[81], remain to be resolved.

to heterogeneous interactions — particularly when disengaged from the MT — which may lead to protein misfolding and aggregation[14].

The MT-binding ability of tau is post-translationally regulated primarily by serine/threonine-directed phosphorylation (FIG. 3), which can effectively modulate the binding affinity of tau for MTs[15]. This is thought to be the most prominent mechanism that regulates the affinity of tau for the MTs[15], although other post-translational modifications, such as glycosylation[16–18], may also have a direct impact on the dynamic equilibrium of tau on and off the MTs (see below). Notably, phosphorylation of tau appears to be developmentally regulated — it is substantially higher during the development of the fetal brain. On the other hand, in the adult brain, neurons are normally characterized by a considerably lower tau phosphorylation state. Furthermore, in the course of tau-mediated neurodegeneration, aberrant tau phosphorylation is always observed (see below). Aside from phosphorylation and glycosylation, other post-translational modifications of tau also occur (see REF. 19 for a recent review), including glycation[20], ubiquitylation[21], sumoylation[22,23], nitration[24] and proteolysis[25]. Although it is conceivable that most or all of these post-translational modifications may take place at various stages of tau pathology, their significance, particularly in comparison to the well-established role of phosphorylation, is yet to be fully characterized.

With its ability to modulate MT-dynamics, tau contributes directly or indirectly to key structural and regulatory cellular functions. For example, the action of tau on the MT network has great importance in maintaining an appropriate morphology of neurons, the processes of which typically extend over relatively great distances, making neurons the most asymmetrical of all cells. Furthermore, because the MT network is key to the sophisticated transport machinery (FIG. 3) that allows signalling molecules, trophic factors and other essential cellular constituents, including organelles (for example mitochondria and vesicles), to travel along the axons (axonal transport), then tau clearly has profound effects on axonal transport and, hence, on the function and viability of neurons and their highly extended processes[26]. Importantly, under normal physiological conditions, tau is in a constant dynamic equilibrium, on and off the MTs. This equilibrium is thought to be controlled primarily by the phosphorylation state of tau, which in turn is determined by the actions of kinases and phosphatases. Indeed, frequent cycles of binding and detachment of tau from the MTs (corresponding to phosphorylations and dephosphorylations, respectively) may be needed to allow effective axonal transport (FIG. 3).

Pathological aggregation of tau

Under pathological conditions, the equilibrium of tau binding to the MTs is perturbed, resulting in an abnormal increase in the levels of the free (unbound) tau

Table 2 | **Therapeutic strategies targeting tau that are currently under investigation**

Therapeutic approach	Expected effect	Current status	Refs
Kinase inhibition	Prevent the abnormal phosphorylation rate or state of tau and consequent excessive disengagement of tau from the MTs	Various stages of preclinical investigation	15,37
Inhibition of tau fibril formation or dissolution of pre-existing aggregates	Prevent aberrantly phosphorylated and/or misfolded tau from forming more organized aggregates	Early stages of drug discovery	67–69,71,99
Activation of chaperone systems	Facilitate the clearance of misfolded tau and/or tau aggregates	Early stages of preclinical investigation	100,101
Stabilization of the MTs	Compensate for the loss of tau's MT-stabilizing function, and thereby sustain axonal transport	Different programmes are at various stages of development, ranging from preclinical investigations to Phase II studies	55,57,102
Aβ-directed therapies	Prevent Aβ from contributing to tau-mediated neurodegeneration	Several compounds in Phase I and Phase II studies	75
Attenuation of inflammation	Attenuation of inflammation might contribute to a slowing of the progression of the disease.	Different programmes are at various stages of development, ranging from early preclinical to Phase III studies	63,103–105

Aβ, amyloid-β; MT, microtubule.

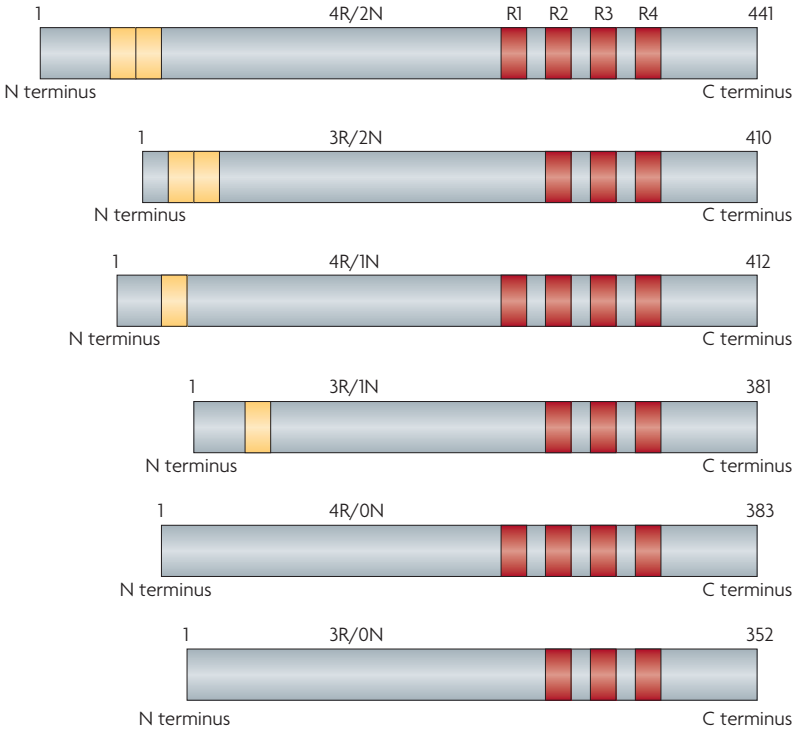

Figure 2 | **The domain structure of the tau isoforms that are expressed in the adult human brain.** The isoforms can differ from each other in the number of tubulin-binding domains (three or four repeats located in the C-terminal half of the protein, shown in red), and are referred to as 3R or 4R tau isoforms, respectively. They can also differ in the presence or absence of either one or two 29-amino-acid-long, highly acidic inserts (shown in yellow) at the N-terminal portion of the protein (the projection domain). Between the projection domain and the microtubule-binding domain lies a basic proline-rich region.

fraction. It is likely that the resultant higher cytosolic concentrations of tau increase the chances of pathogenic conformational changes that in turn lead to the aggregation and fibrillization of tau[14] (FIG. 4). Important progress has been made in recent years in understanding tau misfolding and fibril formation[14,27,28]. The path from normal tau bound to the MTs to large aggregate structures such as NFTs is thought to be a multi-step phenomenon which begins with the detachment of tau from the MTs. The key steps of tau fibrillization are highlighted in FIG. 4. On abnormal disengagement of tau from the MTs, which can be triggered by numerous causes (including increased rate of phosphorylation and/or decreased rate of dephosphorylation), the cytosolic concentration of unbound tau would rise. This is likely to be a crucial step, one which may render tau considerably more likely to undergo misfolding and may, as a result, make it more prone to aggregation. Next, small nonfibrillary tau deposits (normally referred to as 'pretangles') are formed, and these, unlike NFTs, cannot be detected by β-sheet-specific dyes[29–31]. This indicates that pretangles do not contain β-sheets, and that a structural rearrangement involving the formation of the characteristic pleated β-sheet must occur during the transition from pretangles to PHFs. Finally, PHFs further self-assemble to form NFTs.

Causes of tau abnormalities in disease

It is believed that several pathogenic events might contribute, either directly or indirectly, to tau hyperphosphorylation, misfolding and aggregation. Perhaps the most direct cause-and-effect relationship was established by seminal genetic studies that demonstrated that mutations of the tau gene (*MAPT*) are causative of FTD with parkinsonism linked to chromosome-17 (FTDP-17)[32,33]. All cases of FTDP-17 are characterized by the presence of filamentous inclusions that are composed of hyperphosphorylated tau. Such mutations could lead to the expression of tau mutants that are: predisposed to assembly into filaments and therefore able to undergo rapid fibrillization[32,34]; more readily phosphorylated and/or less prone to dephosphorylation[35]; or that show impaired MT binding properties[8,36]. Furthermore, intronic *MAPT* mutations, as well as most coding-region mutations in exon 10 (N279K, L284L, ΔN296, N296N, N296H, S305N and S305S), may alter the alternative splicing of tau to perturb the normal one-to-one ratio of the 3R to 4R tau isoforms, with the 4R isoform being overproduced in most, but not all, instances. Indeed, over 30 different tau gene mutations have been identified in families with FTDP-17 (recently reviewed in REF. 32). Importantly, these genetic studies provide unambiguous evidence that tau malfunction is sufficient to trigger neurodegeneration and dementia even in the absence of other pathogenic insults.

Additional direct cause-and-effect links have been established between tau malfunctions and an overall imbalance in the activity levels or regulation of tau kinases and phosphatases[15,37]. Under physiological conditions, single tau molecules are typically phosphorylated at a subset of potential phosphate-acceptor amino-acid residues. During late stage neurodegeneration, the phosphorylation state of a single tau molecule can reach such high levels that many or most of these residues are phosphorylated and, at the same time, a higher proportion of tau molecules are in this hyperphosphorylated state. Although several kinases have been found to be capable of phosphorylating tau *in vitro*, it is not yet clear whether all of them participate in tau phosphorylation under physiological or pathological conditions *in vivo*[1]. Nonetheless, glycogen synthase kinase 3 (GSK3), cyclin-dependent kinase 5 (CDK5) and the microtubule-affinity-regulating kinase (MARK) have received particular attention as potential targets for disease-modifying therapies using inhibitory compounds[15]. For example, inhibition of GSK3 by lithium not only reduced tau phosphorylation *in vivo*, but also lowered the level of aggregated tau, compared with controls[38]. Other studies on the effects of lithium in transgenic AD mice also suggested a concomitant reduction in Aβ production, possibly resulting from lithium-mediated inhibition of GSK3α, which is required for maximal processing of the precursor of Aβ, amyloid precursor protein (APP)[39]. Similarly, a number of phosphatases[40], including protein phosphatase (PP)1, PP2A, PP2B and PP2C, have been identified that could potentially drive the reverse,

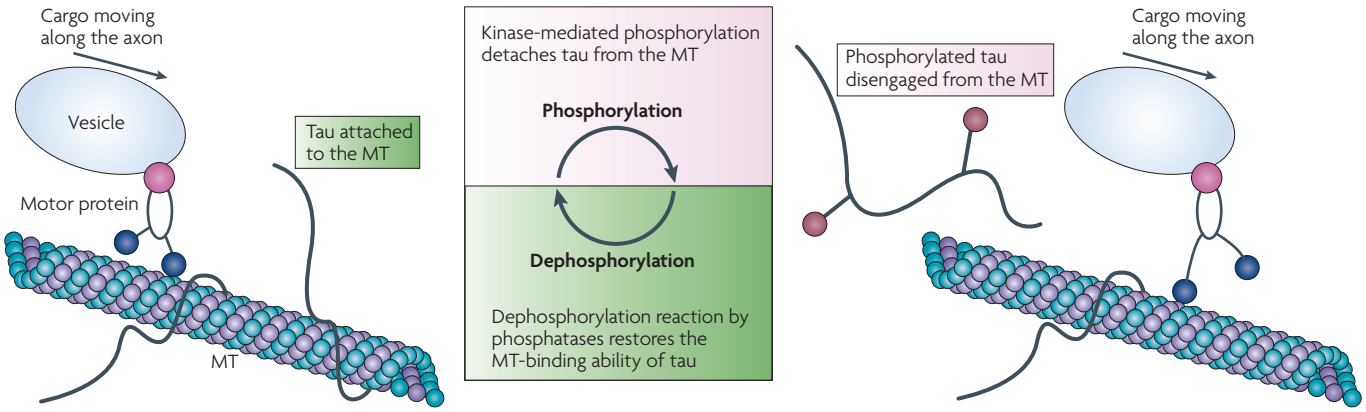

Figure 3 | **The dynamic equilibrium of tau microtubule (MT) binding.** A schematic representation of the normal dynamic equilibrium of tau, on and off the MTs, which is primarily determined by the phosphorylation state of tau. Although the presence of tau on the MTs presents a physical obstacle for vesicles and other cargoes that are moving along the axon, MT-bound tau is essential to MT integrity. Thus, relatively frequent cycles of tau–MT binding (promoted by dephosphorylation of tau) and detachment of tau from the MT (promoted by phosphorylation of tau) are needed in order to maintain effective axonal transport.

dephosphorylation of tau; however, the exact role of these phosphatases under physiological and pathological conditions is not completely clear.

The overall effect of the increased rate and/or state of phosphorylation appears to be the abnormal disengagement of tau from the MTs. Furthermore, it is likely that various other pathological events, including Aβ-mediated toxicity, as well as oxidative stress and inflammation, may be able to trigger or contribute (independently or in combination) to an abnormal detachment of tau from the MTs[41–45]. For example, it has been suggested that oxidative stress could be responsible for detrimental covalent modifications of tau, which include the formation of intermolecular disulphide bridges[46] and tyrosine nitration[24]. Such modifications are likely to cause misfolding, hyperphosphorylation and aggregation, and thereby contribute to abnormal disengagement of tau from MTs, as well as to the formation of aggregates. However, despite the clear involvement of these pathological processes in tau-mediated neurodegeneration, their relative positioning in the cascade of events that leads to neuronal loss remains unclear. For example, although oxidative stress is often regarded as an upstream event relative to tau pathology, recent studies have revealed that pathological tau may interfere with mitochondrial function and induce oxidative stress[47]. This raises the interesting possibility that although oxidative stress is likely to be a relatively early event that could lead to tau malfunction, it is equally possible that tau malfunction, once initiated, may further exacerbate the effects of this, and possibly other, upstream events.

Connections between Aβ-mediated toxicity and tau pathology have repeatedly been proposed[48,49]; however, the mechanism or mechanisms that link SPs and NFTs have not yet been fully established, and this remains one of the most challenging conundrums of AD research. Nonetheless, new lines of investigation support the notion that tau malfunction, in addition to being independently capable of producing neurodegeneration even in the absence of Aβ deposits or other pathological events[32,33], could be a key mediator of neurodegeneration in response to other upstream events, including Aβ-induced toxicity[44]. An interesting and unexpected development of the proposed pathological role of tau as a common mediator of neurodegeneration is the hypothesis that suppression of tau may potentially be beneficial. In accordance with this hypothesis, a recent study[50] has shown that reduction or elimination of endogenous tau, in a mouse model of AD-like Aβ amyloidosis which expresses human amyloid precursor protein (hAPP) with a familial AD mutation that increases Aβ production, is beneficial against Aβ-induced deficits. These results appear to substantiate previous cell-based studies which showed that cultured hippocampal neurons from tau-knockout mice treated with fibrillar Aβ were not susceptible to Aβ-induced toxicity[44]. However, although the most valid model for comparisons with tau suppression would be a tau-knockdown mouse, it is notable that tau-knockout mice show behavioural impairments and structural abnormalities with advancing age[51], suggesting that long-term suppression of tau as a therapy for tauopathies might be fraught with complications.

Tau-mediated neurodegeneration

As described above, in AD and related neurodegenerative disorders that are collectively referred to as tauopathies,[52,53] tau no longer binds to the MTs; instead it becomes sequestered into NFTs in neurons, and into glial tangles in astrocytes or oligodendroglia. In AD at least, the largest burden of tau pathology (~95% of total tau by morphometic analyses) is found in neuronal processes known as neuropil threads or dystrophic neurites[54]. In broad terms, the pathological consequences of these events could result from a loss of normal tau function combined with gains of pathological functions of hyperphosphorylated tau, the filaments formed thereof, and the aggregation of these filaments to form glial and neuronal tangles in dystrophic neurites.

The loss of tau's normal MT-stabilizing function would invariably lead to a pathological disturbance

Oxidative stress
A disturbance in the pro-oxidant–antioxidant balance in favour of the pro-oxidant, leading to potential cellular damage. Indicators of oxidative stress include damaged DNA bases, protein oxidation and lipid peroxidation products.

Dystrophic neurites
The processes (axons and dendrites) of neurons that are damaged or degenerating in AD.

in the normal structural and regulatory functions of the cytoskeleton, which would compromise axonal transport and thus contribute to synaptic dysfunction and neurodegeneration[26,55]. Indeed, the importance of the loss of the MT-stabilizing function of tau in neurodegeneration was recently validated by proof-of-concept studies carried out *in vivo*, which demonstrated that the MT-stabilizing drug paclitaxel can ameliorate the neurodegenerative phenotype of transgenic mouse models of AD-like tau amyloid pathologies[56,57]. However, the discovery that the total level of NFTs correlates with the degree of cognitive impairment[58,59] provided the initial circumstantial evidence to suggest that toxic gains-of-function by NFTs might play an important part in the progression of the disease. Indeed, pioneering studies that used immunohistochemical techniques to determine the level of both NFTs and SPs in different brain regions of AD patients, as well as non-demented elderly individuals, demonstrated that the number of NFTs, but not the numbers of SPs, correlates with the degree of cognitive impairment[58,59].

It is possible that the toxic effects of NFTs may partly arise from the relatively large size of the fibrillary material that accumulates inside the neurons, as this material may pose a direct physical disruption to cellular functions such as axonal transport. Furthermore, NFTs may also contribute to the disease progression by effectively sequestering more tau and other proteins, and thereby reinforcing and amplifying the loss of normal tau function.

However, the notion that NFTs could have a prominent role in the progression of the disease was recently challenged by reports that suppression of transgenic tau in a neurodegenerative tauopathy mouse model produced improvements in memory function, even though NFTs continued to accumulate[60,61]. However, it should be noted that in the model used, the degree of tau suppression is relative to the fully activated state of tau: this means that a 2.5-fold overexpression of tau (compared with endogenous tau) would still be present. Another significant observation was that, in a mouse model of AD-like Aβ pathology, axonal defects that consisted of swellings that contained accumulated abnormal amounts of tau, other proteins and vesicles were found to precede the appearance of Aβ deposits, including SPs, by more than 1 year[62]. Furthermore, restoration of impaired axonal transport in a tauopathy mouse model rescued the disease phenotype[57]. In addition, studies in a transgenic (P301S) tauopathy mouse model revealed that synapse loss and microglial activation precede the appearance of NFTs, presumably due to the impaired transport that results from tau hyperphosphorylation[63].

Collectively, these studies substantiate the notion that axonal transport defects, synapse loss and neuroinflammation may be among the earliest signs of neurodegeneration that results from tau hyperphosphorylation, whereas fibrillary tau tangles may be late-stage manifestations that could contribute to the disease progression by physically interfering with normal cellular functions. At the same time, the tangles may sequester larger quantities of other functionally significant proteins, and thereby exacerbate and amplify upstream causes. It should be noted that the possible relative contribution to neurodegeneration from the toxic gains-of-function versus the loss of normal function may be experimentally difficult to discern, as the toxic gain may imply, at least in part, an amplification of the loss of function. In addition, a precise correlation between the size of the NFTs and these toxic gains (that is, the putative critical mass that is required for an insoluble intracellular deposit to become a physical obstacle) is not yet known.

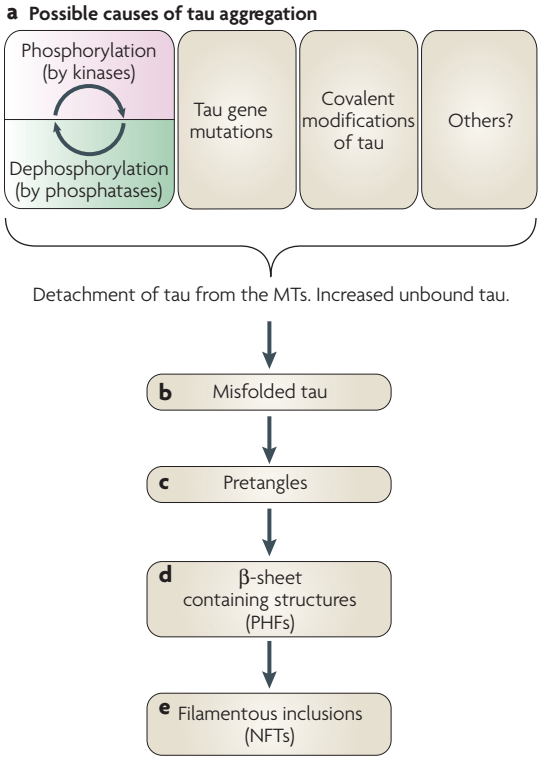

Figure 4 | **Pathological aggregation of tau.** A schematic representation of the different stages of the formation of pathological tau aggregates. **a** | Abnormal disengagement of tau from the MTs and a concomitant increase in the cytosolic concentration of tau are likely to be the key events that lead to tau-mediated neurodegeneration. Direct causes of abnormal disengagement of tau from the MTs include an imbalance of tau kinases and/or phosphatases, mutations of the tau gene, covalent modification of tau causing and/or promoting misfolding, and possibly other causes such as other post-translational modifications. **b** | Once tau is unbound from the MT it becomes more likely to misfold. This is thought to be a stochastic phenomenon that is more likely at higher cytosolic tau concentrations. **c** | Early deposits of tau, called 'pretangles', are not stained by congo red or thioflavine-T, indicating that these intermediate forms of aggregated tau do not exhibit the pleated β-sheet structure typically found in amyloid aggregates. **d** | A structural transition leads to this more organized aggregate and the eventual development of neurofibrillary tangles (**e**). Such transitions may be facilitated by heterogeneous interactions with membranous structures[14,29]. MT, microtubule; NFT, neurofibrillary tangle; PHF, paired helical filament.

Microglia
A non-neuronal cell type that is present in the spinal cord and the brain (it is the resident CNS macrophage) and is characterized by its ramified morphology.

Furthermore, with the existence of pre-fibrillary tau species[30,31], toxic gains-of-function by abnormal tau could be ascribed to one or more of these ill-defined intermediate species. Thus, although loss of tau function and the toxic gain-of-function by PHFs or other abnormal species of tau, in addition to the toxic properties acquired by NFTs as they enlarge, may contribute to neurodegeneration to different extents, it is highly plausible that both types of mechanism contribute to the onset and progression of AD and other tauopathies, especially at different stages of the pathology.

Conclusions and future directions

Despite significant recent advances in our understanding of tau-mediated neurodegeneration, which substantiate the notion that tau may act as a common mediator of neurodegeneration for various upstream pathological events, a detailed picture of causes and effects has not yet emerged. Thus, although it is increasingly clear that the disengagement of tau from MTs is likely to comprise a cardinal step that sets the stage for tau-mediated neurodegeneration, the links between this and other upstream events such as Aβ-mediated toxicity and oxidative stress remain less clear. Likewise, although tau-mediated neurodegeneration probably results from the combination of losses of function and toxic gains-of-function, the specific roles played by the various forms of misfolded and aggregated tau are not fully understood. It should be noted, however, that the onset and progression of the disease may not necessarily be best represented by an unequivocal linear sequence of causes and effects, where one single 'root' cause is responsible for the entire cascade of events. With the recognition that amplification mechanisms exist that could effectively short-circuit cause and effect and thereby exacerbate each-others' detrimental effects, it is plausible that disease progression may lie in the ability to set in motions such self-sustaining cycles. Thus, continuing efforts should be made to further characterize the precise mechanism(s) by which different pathological events may influence and amplify each other. With a wide range of animal models that partially recapitulate the key phenotypic features of AD and related tauopathies already developed[64] (BOX 1; TABLE 1), further insight into the mechanistic roles of the different species of tau aggregates in neurodegenerative disease relies on the discovery of a wider set of molecular tools. These tools must be capable of selectively modulating and/or imaging[65] putative pathogenic and pathological steps. To this end, functional genomics and drug discovery efforts aimed at some of these targets will be instrumental (BOX 2; TABLE 2). For example, agents that are capable of slowing down, blocking or reversing protein tau aggregation *in vitro*[66–74] have been identified; however, issues such as lack of selectivity and/or toxicity have limited their use *in vivo*. In addition, several agents that could compensate for the loss of tau function, such as paclitaxel and other MT-stabilizing agents[55] that have been part of the medical armamentarium for several years, are hampered by limited CNS uptake and toxicities. Drug discovery and lead optimization efforts that could succeed in making these and other agents available for *in vivo* testing will greatly facilitate further understanding of the tau pathway, as well as of the pathogenic significance of specific events, whose exact relation with tau malfunction is less defined.

1. Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A. & Hof, P. R. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res. Rev.* **33**, 95–130 (2000).
2. Brandt, R., Leger, J. & Lee, G. Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. *J. Cell Biol.* **131**, 1327–1340 (1995).
3. Maas, T., Eidenmuller, J. & Brandt, R. Interaction of tau with the neural membrane cortex is regulated by phosphorylation at sites that are modified in paired helical filaments. *J. Biol. Chem.* **275**, 15733–15740 (2000).
4. Fulga, T. A. *et al.* Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration *in vivo*. *Nature Cell Biol.* **9**, 139–148 (2007).
5. Lee, G. Tau and src family tyrosine kinases. *Biochim. Biophys. Acta* **1739**, 323–330 (2005).
6. Lee, G., Neve, R. L. & Kosik, K. S. The microtubule binding domain of tau protein. *Neuron* **2**, 1615–1624 (1989).
7. Binder, L. I., Frankfurter, A. & Rebhun, L. I. The distribution of tau in the mammalian central nervous system. *J. Cell Biol.* **101**, 1371–1378 (1985).
8. Hong, M. *et al.* Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. *Science* **282**, 1914–1917 (1998).
9. Amos, L. A. Microtubule structure and its stabilisation. *Org. Biomol. Chem.* **2**, 2153–2160 (2004).
10. Kar, S., Fan, J., Smith, M. J., Goedert, M. & Amos, L. A. Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. *EMBO J.* **22**, 70–77 (2003).
11. Kar, S., Florence, G. J., Paterson, I. & Amos, L. A. Discodermolide interferes with the binding of tau protein to microtubules. *FEBS Lett.* **539**, 34–36 (2003).
12. Kampers, T., Pangalos, M., Geerts, H., Wiech, H. & Mandelkow, E. Assembly of paired helical filaments from mouse tau: implications for the neurofibrillary pathology in transgenic mouse models for Alzheimer's disease. *FEBS Lett.* **451**, 39–44 (1999).
13. Takashima, A. *et al.* Presenilin 1 associates with glycogen synthase kinase-3β and its substrate tau. *Proc. Natl Acad. Sci. USA* **95**, 9637–9641 (1998).
14. Kuret, J. *et al.* Evaluating triggers and enhancers of tau fibrillization. *Microsc. Res. Tech.* **67**, 141–155 (2005). **This review provides a model to rationalize the multistep pathway to tau fibril formation, as well as experimental methods for tau fibrillization assays.**
15. Mazanetz, M. P. & Fischer, P. M. Untangling tau hyperphosphorylation in drug design for neurodegenerative diseases. *Nature Rev. Drug Discov.* **6**, 464–479 (2007). **An up-to-date account of the role of specific kinases in tau-mediated neurodegeneration and their significance as targets for therapeutic intervention.**
16. Arnold, C. S. *et al.* The microtubule-associated protein tau is extensively modified with *O*-linked *N*-acetylglucosamine. *J. Biol. Chem.* **271**, 28741–28744 (1996).
17. Li, X., Lu, F., Wang, J.-Z. & Gong, C.-X. Concurrent alterations of *O*-GlcNAcylation and phosphorylation of tau in mouse brains during fasting. *Eur. J. Neurosci.* **23**, 2078–2086 (2006).
18. Liu, F., Iqbal, K., Grundke-Iqbal, I., Hart, G. W. & Gong, C.-X. *O*-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **101**, 10804–10809 (2004).
19. Gong, C. X., Liu, F., Grundke-Iqbal, I. & Iqbal, K. Post-translational modifications of tau protein in Alzheimer's disease. *J. Neural Transm.* **112**, 813–838 (2005).
20. Münch, G., Deuther-Conrad, W. & Gasic-Milenkovic, J. Glycoxidative stress creates a vicious cycle of neurodegeneration in Alzheimer's disease – a target for neuroprotective treatment strategies? *J. Neural Transm.* **62** (Suppl.), 303–307 (2002).
21. Cripps, D. *et al.* Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. *J. Biol. Chem.* **281**, 10825–10838 (2006).
22. Dorval, V. & Fraser, P. E. Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and α-synuclein. *J. Biol. Chem.* **281**, 9919–9924 (2006).
23. Dorval, V. & Fraser, P. E. SUMO on the road to neurodegeneration. *Biochim. Biophys. Acta* **1773**, 694–706 (2007).
24. Mailliot, C., Trojanowski, J. Q. & Lee, V. M. Impaired tau protein function following nitration-induced oxidative stress *in vitro* and *in vivo*. *Neurobiol. Aging* **23** (Suppl. 1), 415 (2002).
25. Johnson, G. Tau phosphorylation and proteolysis: insights and perspectives. *J. Alzheimers Dis.* **9**, 243–250 (2006).
26. Roy, S., Zhang, B., Lee, V. M.-Y. & Trojanowski, J. Q. Axonal transport defects: a common theme in neurodegenerative diseases. *Acta Neuropathol.* **109**, 5–13 (2005). **Reviews the biology of axonal transport and its role in neurodegenerative disease.**
27. Kuret, J. *et al.* Pathways of tau fibrillization. *Biochim. Biophys. Acta* **1739**, 167–178 (2005).
28. Ross, C. A. & Poirier, M. A. Protein aggregation and neurodegenerative disease. *Nature Med.* **10**, S10–S17 (2004).
29. Galvan, M., David, J. P., Delacourte, A., Luna, J. & Mena, R. Sequence of neurofibrillary changes in aging and Alzheimer's disease: a confocal study with phospho-tau antibody, AD2. *J. Alzheimers Dis.* **3**, 417–425 (2001).

30. Maeda, S. *et al.* Granular tau oligomers as intermediates of tau filaments. *Biochemistry* **46**, 3856–3861 (2007).
31. Maeda, S. *et al.* Increased levels of granular tau oligomers: an early sign of brain aging and Alzheimer's disease. *Neurosci. Res.* **54**, 197–201 (2006).
32. Goedert, M. & Jakes, R. Mutations causing neurodegenerative tauopathies. *Biochim. Biophys. Acta* **1739**, 240–250 (2005).
33. von Bergen, M. *et al.* Mutations of tau protein in frontotemporal dementia promote aggregation of paired helical filaments by enhancing local β-structure. *J. Biol. Chem.* **276**, 48165–48174 (2001).
34. Nacharaju, P. *et al.* Accelerated filament formation from tau protein with specific FTDP-17 missense mutations. *FEBS Lett.* **447**, 195–199 (1999).
35. Alonso Adel, C., Mederlyova, A., Novak, M., Grundke-Iqbal, I. & Iqbal, K. Promotion of hyperphosphorylation by frontotemporal dementia tau mutations. *J. Biol. Chem.* **279**, 34873–34881 (2004).
36. Dayanandan, R. *et al.* Mutations in tau reduce its microtubule binding properties in intact cells and affect its phosphorylation. *FEBS Lett.* **446**, 228–232 (1999).
37. Churcher, I. Tau therapeutic strategies for the treatment of Alzheimer's disease. *Curr. Top. Med. Chem.* **6**, 579–595 (2006).
38. Noble, W. *et al.* Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration *in vivo*. *Proc. Natl Acad. Sci. USA* **102**, 6990–6995 (2005).
Study that validates GSK3β as a target for tau-directed therapies.
39. Phiel, C. J., Wilson, C. A., Lee, V. M. Y. & Klein, P. S. GSK-3α regulates production of Alzheimer's disease amyloid-β peptides. *Nature* **423**, 435–439 (2003).
40. Tian, Q. & Wang, J. Role of serine/threonine protein phosphatase in Alzheimer's disease. *Neurosignals* **11**, 262–269 (2002).
41. Andersen, J. K. Oxidative stress in neurodegeneration: cause or consequence? *Nature Med.* **5**, S18–S25 (2004).
42. Moreira, P. I. *et al.* Oxidative stress and neurodegeneration. *Ann. NY Acad. Sci.* **1043**, 545–552 (2005).
43. King, M. E. *et al.* Tau-dependent microtubule disassembly initiated by prefibrillar β-amyloid. *J. Cell Biol.* **175**, 541–546 (2006).
44. Rapoport, M., Dawson, H. N., Binder, L. I., Vitek, M. P. & Ferreira, A. Tau is essential to β-amyloid-induced neurotoxicity. *Proc. Natl Acad. Sci. USA* **99**, 6364–6369 (2002).
Study that substantiates the notion that tau may be a mediator of upstream pathological events, namely Aβ-induced neurotoxicity.
45. Liu, Q. *et al.* Tau modifiers as therapeutic targets for Alzheimer's disease. *Biochim. Biophys. Acta* **1739**, 211–215 (2005).
46. Schweers, O., Mandelkow, E., Biernat, J. & Mandelkow, E. Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein τ controls the *in vitro* assembly of paired helical filaments. *Proc. Natl Acad. Sci. USA* **92**, 8463–8467 (1995).
47. David, D. C. *et al.* Proteomic and functional analyses reveal a mitochondrial dysfunction in P301L tau transgenic mice. *J. Biol. Chem.* **280**, 23802–23814 (2005).
48. Blurton-Jones, M. & LaFerla, F. M. Pathways by which Aβ facilitates tau pathology. *Curr. Alzheimer Res.* **3**, 437–448 (2006).
49. Oddo, S. *et al.* Temporal profile of amyloid-β (Aβ) oligomerization in an *in vivo* model of Alzheimer disease: a link between Aβ and tau pathology. *J. Biol. Chem.* **281**, 1599–1604 (2006).
50. Roberson, E. D. *et al.* Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. *Science* **316**, 750–754 (2007).
Study that suggests the role tau might have as a mediator of neurodegeneration. This study also suggests that tau reduction may be therapeutically beneficial.
51. Ikegami, S., Harada, A. & Hirokawa, N. Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice. *Neurosci. Lett.* **279**, 129–132 (2000).
52. Forman, M. S., Trojanowski, J. Q. & Lee, V. M.-Y. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. *Nature Med.* **10**, 1055–1063 (2004).
53. Trojanowski, J. Q. & Mattson, M. P. Overview of protein aggregation in single, double, and triple neurodegenerative brain amyloidoses. *Neuromolecular Med.* **4**, 1–6 (2003).
54. Mitchell, T. W. *et al.* Novel method to quantify neuropil threads in brains from elders with or without cognitive impairment. *J. Histochem. Cytochem.* **48**, 1627–1638 (2000).
55. Trojanowski, J. Q., Smith, A. B., Huryn, D. & Lee, V. M.-Y. Microtubule-stabilizing drugs for therapy of Alzheimer's disease and other neurodegenerative disorders with axonal transport impairments. *Expert Opin. Pharmacother.* **6**, 683–686 (2005).
56. Ishihara, T. *et al.* Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* **24**, 751–762 (1999).
57. Zhang, B. *et al.* Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proc. Natl Acad. Sci. USA* **102**, 227–231 (2005).
58. Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T. & Hyman, B. T. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639 (1992).
59. Arriagada, P. V., Marzloff, K. & Hyman, B. T. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology* **42**, 1681–1688 (1992).
60. Santacruz, K. *et al.* Tau suppression in a neurodegenerative mouse model improves memory function. *Science* **309**, 476–481 (2005).
Interesting study that shows that suppression of tau improves memory function even though NFTs continue to grow.
61. Trojanowski, J. Q. & Lee, V. M. Pathological tau: a loss of normal function or a gain in toxicity? *Nature Neurosci.* **8**, 1136–1137 (2005).
62. Stokin, G. B. *et al.* Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science* **307**, 1282–1288 (2005).
Together with reference 61, this study shows that axonal transport defects may be early pathological events in tau-mediated neurodegeneration.
63. Yoshiyama, Y. *et al.* Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* **53**, 337–351 (2007).
Paper demonstrating that microgliosis and synaptic pathology may be the earliest manifestation of neurodegenerative tauopathies. The paper also suggests that abrogation of tau-induced microglial activation may be therapeutically beneficial.
64. Lee, V. M.-Y., Kenyon, T. K. & Trojanowski, J. Q. Transgenic animal models of tauopathies. *Biochim. Biophys. Acta* **1739**, 251–259 (2005).
65. Shaw, L. M., Korecka, M., Clark, C. M., Lee, V. M. & Trojanowski, J. Q. Biomarkers of neurodegeneration for diagnosis and monitoring therapeutics. *Nature Rev. Drug Discov.* **6**, 295–303 (2007).
66. Necula, M., Chirita, C. N. & Kuret, J. Cyanine dye N744 inhibits tau fibrillization by blocking filament extension: implications for the treatment of tauopathic neurodegenerative diseases. *Biochemistry* **44**, 10227–10237 (2005).
67. Pickhardt, M. *et al.* Screening for inhibitors of tau polymerization. *Curr. Alzheimer Res.* **2**, 219–226 (2005).
68. Pickhardt, M. *et al.* Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments *in vitro* and in cells. *J. Biol. Chem.* **280**, 3628–3635 (2005).
69. Taniguchi, S. *et al.* Inhibition of heparin-induced tau filament formation by phenothiazines, polyphenols, and porphyrins. *J. Biol. Chem.* **280**, 7614–7623 (2005).
70. Frid, P., Anisimov, S. V. & Popovic, N. Congo red and protein aggregation in neurodegenerative diseases. *Brain Res. Rev.* **53**, 135–160 (2007).
71. Chirita, C., Necula, M. & Kuret, J. Ligand-dependent inhibition and reversal of tau filament formation. *Biochemistry* **43**, 2879–2887 (2004).
72. Liu, M., Ni, J., Kosik, K. S. & Yeh, L. A. Development of a fluorescent high throughput assay for tau aggregation. *Assay Drug Dev. Technol.* **2**, 609–619 (2004).
73. Wischik, C. M., Edwards, P. C., Lai, R. Y. K., Roth, M. & Harrington, C. R. Selective inhibition of Alzheimer disease-like tau aggregation by phenothiazines. *Proc. Natl Acad. Sci. USA* **93**, 11213–11218 (1996).
74. Ignatova, Z. & Gierasch, L. M. Inhibition of protein aggregation *in vitro* and *in vivo* by a natural osmoprotectant. *Proc. Natl Acad. Sci. USA* **103**, 13357–13361 (2006).
75. Gotz, J. *et al.* A decade of tau transgenic animal models and beyond. *Brain Pathol.* **17**, 91–103 (2007).
An up-to-date account of tau transgenic animal models.
76. McGowan, E., Eriksen, J. & Hutton, M. A decade of modeling Alzheimer's disease in transgenic mice. *Trends Genet.* **22**, 281–289 (2006).
77. Van Dam, D. & De Deyn, P. P. Drug discovery in dementia: the role of rodent models. *Nature Rev. Drug Discov.* **5**, 956–970 (2006).
78. Schindowski, K. *et al.* Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. *Am. J. Pathol.* **169**, 599–616 (2006).
79. Melnikova, I. Therapies for Alzheimer's disease. *Nature Rev. Drug Discov.* **6**, 341–342 (2007).
80. Okamura, N. *et al.* Quinoline and benzimidazole derivatives: candidate probes for *in vivo* imaging of tau pathology in Alzheimer's disease. *J. Neurosci.* **25**, 10857–10862 (2005).
81. Pardridge, W. M. The blood–brain barrier: bottleneck in brain drug development. *NeuroRx* **2**, 3–14 (2005).
82. Gotz, J. *et al.* Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform. *EMBO J.* **14**, 1304–1313 (1995).
83. Spittaels, K. *et al.* Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein. *Am. J. Pathol.* **155**, 2153–2165 (1999).
84. Probst, A. *et al.* Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. *Acta Neuropathol.* **99**, 469–481 (2000).
85. Ishihara, T. *et al.* Age-dependent induction of congophilic neurofibrillary tau inclusions in tau transgenic mice. *Am. J. Pathol.* **158**, 555–562 (2001).
86. Lewis, J. *et al.* Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nature Genet.* **25**, 402–405 (2000).
87. Gotz, J., Chen, F., Barmettler, R. & Nitsch, R. M. Tau filament formation in transgenic mice expressing P301L tau. *J. Biol. Chem.* **276**, 529–534 (2001).
88. Duff, K. *et al.* Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes. *Neurobiol. Dis.* **7**, 87–98 (2000).
89. Gotz, J. *et al.* Oligodendroglial tau filament formation in transgenic mice expressing G272V tau. *Eur. J. Neurosci.* **13**, 2131–2140 (2001).
90. Allen, B. *et al.* Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. *J. Neurosci.* **22**, 9340–9351 (2002).
91. Lim, F. *et al.* FTDP-17 mutations in tau transgenic mice provoke lysosomal abnormalities and tau filaments in forebrain. *Mol. Cell Neurosci.* **18**, 702–714 (2001).
92. Tanemura, K. *et al.* Neurodegeneration with tau accumulation in a transgenic mouse expressing V337M human tau. *J. Neurosci.* **22**, 133–141 (2002).
93. Tatebayashi, Y. *et al.* Tau filament formation and associative memory deficit in aged mice expressing mutant (R406W) human tau. *Proc. Natl Acad. Sci. USA* **99**, 13896–13901 (2002).
94. Tesseur, I. *et al.* Prominent axonopathy and disruption of axonal transport in transgenic mice expressing human apolipoprotein E4 in neurons of brain and spinal cord. *Am. J. Pathol.* **157**, 1495–1510 (2000).
95. Ahlijanian, M. K. *et al.* Hyperphosphorylated tau and neurofilament and cytoskeletal disruptions in mice overexpressing human p25, an activator of cdk5. *Proc. Natl Acad. Sci. USA* **97**, 2910–2915 (2000).

96. Capsoni, S. *et al.* Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. *Proc. Natl Acad. Sci. USA* **97**, 6826–6831 (2000).
97. Oddo, S., Caccamo, A., Kitazawa, M., Tseng, B. P. & LaFerla, F. M. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol. Aging* **24**, 1063–1070 (2003).
98. Oddo, S. *et al.* Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* **39**, 409–421 (2003).
99. Crowe, A., Ballatore, C., Hyde, E., Trojanowski, J. Q. & Lee, V. M.-Y. High throughput screening for small molecule inhibitors of heparin-induced tau fibril formation. *Biochem. Biophys. Res. Commun.* **358**, 1–6 (2007).
100. Dickey, C. A. *et al.* The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. *J. Clin. Invest.* **117**, 648–658 (2007).
101. Goryunov, D. & Liem, R. K. H. CHIP-ping away at tau. *J. Clin. Invest.* **117**, 590–592 (2007).
102. Matsuoka, Y. *et al.* Intranasal NAP administration reduces accumulation of amyloid peptide and tau hyperphosphorylation in a transgenic mouse model of Alzheimer's disease at early pathological stage. *J. Mol. Neurosci.* **31**, 165–170 (2007).
103. Pasinetti, G. M. From epidemiology to therapeutic trials with anti-inflammatory drugs in Alzheimer's disease: the role of NSAIDs and cyclooxygenase in β-amyloidosis and clinical dementia. *J. Alzheimers Dis.* **4**, 435–445 (2002).
104. Klegeris, A. & McGeer, P. L. Non-steroidal anti-inflammatory drugs (NSAIDs) and other anti-inflammatory agents in the treatment of neurodegenerative disease. *Curr. Alzheimer Res.* **2**, 355–365 (2005).
105. Townsend, K. P. & Pratico, D. Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs. *FASEB J.* **19**, 1592–1601 (2005).

Acknowledgements
We thank our colleagues for their contributions to the work summarized here, which has been supported by grants from the US National Institutes of Health (P01 AG09215, P30 AG10124, P01 AG11542, P01 AG14382, P01 AG14449, P01 AG17586, PO1 AG19724, P01 NS-044233, UO1 AG24904), and the Marian S. Ware Alzheimer Program. Finally, we are indebted to our patients and their families, whose commitment to research has made our work possible.

Competing interests statement
The authors declare no competing financial interests.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
APP | CDK5 | FYN | GSK3α | GSK3β | MAPT | MARK | presenilin 1
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer's disease

FURTHER INFORMATION

John Q. Trojanowski's homepages:
http://www.med.upenn.edu/aging
http://www.uphs.upenn.edu/ADC
http://www.uphs.upenn.edu/cndr/
ALL LINKS ARE ACTIVE IN THE ONLINE PDF.