Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1739 (2005) 280–297
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Review

Tau phosphorylation: physiological and pathological consequences

William H. Stoothoff, Gail V.W. Johnson*

Department of Psychiatry, School of Medicine, University of Alabama at Birmingham, 1061 Sparks Center, 1720 7th Avenue South, Birmingham, AL 35294-0017, USA

Received 7 May 2004; accepted 2 June 2004
Available online 23 July 2004

Abstract

The microtubule-associated protein tau, abundant in neurons, has gained notoriety due to the fact that it is deposited in cells as fibrillar lesions in numerous neurodegenerative diseases, and most notably Alzheimer's disease. Regulation of microtubule dynamics is the most well-recognized function of tau, but it is becoming increasingly evident that tau plays additional roles in the cell. The functions of tau are regulated by site-specific phosphorylation events, which if dysregulated, as they are in the disease state, result in tau dysfunction and mislocalization, which is potentially followed by tau polymerization, neuronal dysfunction and death. Given the increasing evidence that a disruption in the normal phosphorylation state of tau plays a key role in the pathogenic events that occur in Alzheimer's disease and other neurodegenerative conditions, it is of crucial importance that the protein kinases and phosphatases that regulate tau phosphorylation in vivo as well as the signaling cascades that regulate them be identified. This review focuses on recent literature pertaining to the regulation of tau phosphorylation and function in cell culture and animal model systems, and the role that a dysregulation of tau phosphorylation may play in the neuronal dysfunction and death that occur in neurodegenerative diseases that have tau pathology.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Tau; Tauopathy; Phosphorylation; Filament; Kinase; Phosphatase

1. Introduction

Tau is a family of neuronal proteins that are produced by alternative mRNA splicing of a single gene. The most well-defined role of tau in the cell is to promote microtubule stability; however, it is becoming increasing evident that tau has many other functions (for a review, see Ref. [1]). The functions of tau are regulated by its phosphorylation state, and tau is a champion when it comes to phosphorylation. The longest form of human brain tau contains 80 Ser or Thr residues and five Tyr residues, and therefore almost 20% of the protein has the potential to be phosphorylated. In vitro, dozens of protein kinases have been shown to phosphorylate tau; indeed, tau maybe considered a "universal phosphate acceptor". Although innumerable protein kinases can phosphorylate tau in vitro, the number that regulates the phosphorylation state of tau in a physiologically relevant context is significantly lower. Therefore, to limit the scope of this review, only tau phosphorylation events that occur in situ or in vivo will be discussed after a brief history of tau.

2. A brief history of tau

Tau was first discovered in 1975 by Weingarten et al. [2] as a protein that co-purified with microtubules through cycles of assembly and disassembly and was called tau for its ability to induce tubule formation. In 1977 it was noted that tau was a phosphoprotein [3], and in 1984 it was demonstrated that tau in a more dephosphorylated state is more efficient at promoting microtubule assembly [4].

* Corresponding author. Tel.: +1 205 934 2465; fax: +1 205 934 2500.
E-mail address: gvwj@uab.edu (G.V.W. Johnson).

0925-4439/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2004.06.017

Subsequent to these findings, there was only moderate interest in tau until it was demonstrated that tau made up the paired helical filaments (PHFs) which form the neurofibrillary tangles (NFTs) found in Alzheimer's disease brain and that the tau that formed these filaments was abnormally phosphorylated [5–7]. These findings catapulted tau into the limelight, and studies on the phosphorylation of tau increased exponentially. However, interest in the mechanisms regulating tau phosphorylation seemed to dwindle when it was discovered that mutations in genes that affect the production of Aβ (the peptide that forms the core of the amyloid plaques that make up the other predominant pathological lesion in Alzheimer's disease brain) caused familial Alzheimer's disease [8,9]. These findings, along with others demonstrating the toxicity and pathogenic properties of Aβ (reviewed in Ref. [10]), lead to the notion that tau pathology was not central to the disease and therefore there was less interest in tau and tau phosphorylation. However, the tau field was re-energized when it was discovered that mutations in the tau gene caused rare autosomal dominant neurodegenerative diseases [collectively known as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)], in which there was pronounced tau pathology but no amyloid pathology [11–14], and the tau in FTDP-17 brains was also abnormally phosphorylated. Further, there was a growing list of sporadic neurodegenerative diseases that were termed "tauopathies" as abnormal lesions containing phosphorylated tau were present in the brain again without significant amyloid pathology (reviewed in Ref. [15]). Overall, these findings clearly indicate that alterations in tau phosphorylation and function likely play a significant role in a variety of neurodegenerative diseases, and therefore understanding the mechanisms that regulate tau phosphorylation and the outcome of these events is imperative.

3. Tau phosphorylation in non-mammalian models

Despite the renewed interest in tau phosphorylation, function, and dysfunction, a suitable animal model that accurately recapitulates the tau pathology and neuronal degeneration found in human tauopathies has not been established. While the models that have been established may not be completely accurate in their representation of human disease states, they do provide valuable insights into how tau functions in normal and pathological states, and the role that phosphorylation plays in modulating tau function. In this section, non-mammalian models used to analyze tau phosphorylation and function will be presented.

3.1. Tau in the worm

The simplest animal model that has been established to examine tau function to date is the nematode *Caenorhabditis elegans*. While these nematodes have a very primitive nervous system (no brain!), they do possess large, easily visible neurons, which have been mapped and provide a good in vivo environment to examine tau localization and function. Although it is relatively easy to manipulate *C. elegans* genetically, and it has been used to study the function of a number of neuronal proteins (reviewed in Ref. [16]), only one recent study has utilized this model to investigate the functions and effects of wild-type and FTDP-17 mutant tau on neuronal function [17]. In this study, it was demonstrated that overexpression of wild-type or FTDP-17 mutant tau (V337M or P301L) decreased the life span and the motility of the worms. Tau overexpression in *C. elegans* also resulted in a presynaptic deficit in cholinergic transmission. Although in this model both wild-type and FTDP-17 mutant tau were phosphorylated, and over time there was an increase in insoluble tau, there was not a clear-cut association between tau phosphorylation/aggregation and the functional/behavioral deficits [17].

3.2. Tau in the fly

Similar to *C. elegans*, the fruit fly (*Drosophila melanogaster*) is a convenient and powerful model that can be used to examine tau phosphorylation and function in the context of the nervous system in an intact animal. While the nervous system of a fly is still quite simplistic (there is no true brain or central nervous system), it is more complex than that of the worm. One powerful advantage of using *Drosophila* is the fact that every neuron has been mapped, both developmentally and in relation to its connections in the adult animal [18,19]. This provides an excellent model to determine the effects of tau on cell migration, axon pathfinding, synaptogenesis, and cellular function.

Drosophila express a microtubule-associated protein (MAP) that promotes microtubule assembly and migrates on SDS-polyacrylamide gels with an approximate molecular weight of 85 kDa. In addition, this protein, termed DMAP-85, is recognized by certain tau-specific antibodies [20]. Like mammalian tau, DMAP-85 is also functionally regulated by phosphorylation and localizes to axons of the ventral nerve cord. These results show that tau-like proteins are present in lower animals and perform similar functions to tau, suggesting that tau function is evolutionarily important and conserved.

Panneuronal expression of both wild-type and FTDP-17 (R406W) mutant tau significantly decreased the life span of the flies, although expression of the R406W tau resulted in a greater decrease in life span than wild-type tau [21]. Similar to the results of a previous study [22], the authors found that expression of wild-type human tau caused neurodegeneration in the tau-expressing cells. Overexpression of the tau constructs did not alter the developmental progression of neurons or axon pathfinding. In contrast,

aged tau transgenic flies showed increased neurodegeneration compared to nontransgenic flies, with the flies expressing R406W tau showing a more severe phenotype. Further, phosphotau immunohistochemical staining increased with age in both the wild-type- and R406W tau-expressing flies, and the immunoreactivity localized to degenerating neurons. However, no immunoblots were shown to demonstrate increased tau phosphorylation in the tau transgenic flies. Additionally, electron microscopic examination of transgenic fly brains and sarkosyl insoluble fractions revealed no neurofibrillary pathology, even in flies expressing the R406W mutant tau [21]. Results from this study demonstrate that overexpression of wild-type or mutant human tau in fly neurons is sufficient to cause age-dependent degeneration of neurons, and this neurodegeneration is not dependent on the formation of intracellular tau aggregates. In this model, as in the other tau overexpression animal models, it would be interesting to determine if the neurodegenerative effects are specific for tau, or if overexpression of another MAP, such as MAP-2, also results in neuronal degeneration. These experiments are important to validate the usefulness of tau overexpression models to examine the mechanisms responsible for the neuronal degeneration that occurs in the human disease states, as increased tau expression is not a feature of the human tauopathies.

In a subsequent study, both tau and a protein kinase that phosphorylates tau, glycogen synthase kinase 3β (GSK3β), were overexpressed in fly neurons [23]. As observed previously, overexpression of wild-type human tau in neurons resulted in an increase in apoptotic markers and cell death. Expression of GSK3β together with tau resulted in an enhanced toxicity. Interestingly, co-expression of tau and GSK3β produced large intracellular tau-containing aggregates that contained filaments, although a robust increase in tau phosphorylation was not demonstrated [23]. While these findings are intriguing, the increased toxicity in response to the expression of GSK3β with tau may not be due to an increase in phosphorylation of tau by GSK3β, but may be due to the fact that GSK3β is a pro-apoptotic protein (for a review, see Ref. [24]), and hence increases the vulnerability of the neurons to the toxic effect of tau overexpression.

Shulman and Feany [25] used a genetic screen in *Drosophila* to identify unique modifiers of human tau toxicity. Screening nearly 2300 different genes, they identified 24 genes that enhanced or suppressed the neuronal toxicity of tau. The majority of the identified clones were *Drosophila* homologs of kinases and phosphatases, which could directly or indirectly modulate the phosphorylation state of tau. Some phosphatases and kinases shown to enhance tau-induced neuronal degeneration are protein phosphatase (PP) 2A subunit B, center divider kinase, and Tao1 (fly homolog of STE20-related kinase). Kinase/phosphatase suppressors identified were MARK/PAR-1, two CDC-25 phosphatase homologs (string and twine), a PP1 subunit, and the phosphatidic acid phosphatase. Interestingly, GSK3β, c-jun N-terminal kinase (JNK), and cyclin dependent kinase-5 (cdk5), all of which have been shown to phosphorylate tau in vitro [26–30], were not identified by the assay as modifiers of tau toxicity. Nonetheless, these findings suggest that phosphorylation events play a role in regulating the toxicity of tau when it is exogenously expressed.

3.3. Tau in the lamprey

The sea lamprey (*Petromyzon marinus*) is a jawless, primitive fish with a notochord that has been used to examine the effects of tau overexpression on neuronal survival in an intact vertebrate animal. The lamprey has several giant, easily identified reticulospinal neurons [anterior bulbar cells (ABCs)], which can be made to express human proteins by microinjecting the appropriate mRNA, and analyzed over a time course (reviewed in Ref. [31]). One group has exploited this model system to examine the effects of tau expression and phosphorylation on cellular function. In 1997, Hall et al. [32] demonstrated that when human tau is overexpressed in lamprey ABCs, it becomes phosphorylated and forms filamentous inclusions. The authors showed tau localized throughout the cell, including the soma and dendrites. When they examined the distribution of tau phosphorylated at the PHF-1 ($Ser^{396/404}$) and Tau-1 (Thr^{198}-Ser^{205}) epitopes, they found it was predominantly in the dendrites, in contrast to a primarily axonal localization of tau as it is in human brain [1]. However, even though tau localized primarily to the dendrites, there were filamentous tau inclusions and evidence of degenerative changes, indicating that tau's toxicity is not dependent upon axonal localization. This group went on to demonstrate that that cellular changes, such as cytoskeletal disruption and membrane degeneration, are spatially and temporally correlated with the appearance and progressive formation, of intracellular tau straight filaments [33]. Interestingly when purified PHFs from Alzheimer's disease brain were injected into the ABCs, they were dephosphorylated and proteolyzed within 2 days, indicating that there maybe a malfunction of normal dephosphorylation and/or proteolytic mechanisms in Alzheimer's disease brain [34]. In a more recent study, it was demonstrated that proprietary compounds, which inhibit tau filament formation in vitro, also inhibited tau filament formation in lamprey ABCs and attenuated the degenerative changes that occur in response to tau overexpression [35]. These data suggest that in the lamprey, tau filaments are toxic to the cells. Overall, these studies in the lamprey have contributed to our understanding of the possible pathogenic outcomes of tau overexpression. Interestingly, the lamprey was the first in vivo model of tau filament formation [32]. Given this fact, it is unfortunate that up to this point no other group has used the lamprey as a model system to elucidate the

sequence of events that eventually lead to tau filament formation.

3.4. Tau in the zebrafish

Zebrafish (*Danio rerio*) have been used to elucidate numerous aspects of development such as signaling pathways involved in organogenesis and embryonic patterning, and have also provided a model for several human diseases (reviewed in Refs. [36–38]). In spite of the well-characterized biology of the zebrafish, and this model system's usefulness in elucidating the pathogenic underpinnings of several human diseases, only one study has attempted to use this model to study the tauopathies.

In 2002, Tomasiewicz et al. [39] demonstrated that the zebrafish could be genetically engineered to overexpress human tau resulting in the formation of filamentous structures. Further, the exogenously expressed human tau is phosphorylated in this in vivo model system. Interestingly, in this model system, the expressed tau redistributes, in a time-dependent manner, from the axon to the soma where tau-positive filaments form. In contrast to some other studies, in this model system exogenously expressed three-repeat tau did not form inclusions or induce cytoskeletal disruption. Finally, the authors demonstrate that expression of tau in neurons leads to phosphorylation of tau on PHF-tau immunoreactive sites (PHF-1), and that GSK3β is expressed in fish brain [39]. These results establish the usefulness of the zebrafish as a possible model system for the study of human tauopathies.

3.5. Tau in the frog

The *Xenopus laevis* oocyte is another popular model system frequently used to examine or model developmental processes. *Xenopus* has also been widely used as a good model for electrophysiological studies, due to the fact that oocytes will express functional ion channels when the mRNA is microinjected. However, like most other non-mammalian model systems, *Xenopus* has not been frequently used as a model to study tau-toxicity or tauopathy. However, in 2002 one group published three reports, all pertaining to tau phosphorylation and mutant tau effects using *Xenopus* as the model system [40–42]. Two studies examined the phosphorylation of exogenously expressed tau, one with respect to protein kinase A (PKA) and GSK3β [41] and the other in the context of mitosis [40]. *Xenopus* oocytes can be induced to mature by the application of progesterone. During prophase I, both PKA and GSK3β are active, while induction with progesterone causes inactivation of both and activation of numerous other kinases [e.g., cdk2 and mitogen activated protein kinase (MAPK or ERK2)] during meiosis I [41]. During prophase, tau phosphorylation at the AD2 ($Ser^{396/404}$) site increased in a time-dependent manner, which was blocked by LiCl, a GSK3β inhibitor, while induction with progesterone increased phosphorylation at this epitope in a LiCl-independent manner. Phosphorylation at the AT100 (Thr^{212}/Ser^{214}) epitope was not inhibited by LiCl, and expression of exogenous PKA led to phosphorylation at both the AD2 and AT100 epitopes in a LiCl-independent (and therefore GSK3β independent) manner [40,41]. In the context of mitosis [40], the authors examined tau phosphorylation at the AD2, Tau-1, TG3 (Thr^{231}), and AT100 epitopes. By 4 h post-progesterone induction, phosphorylation was significantly increased at all sites examined. Injection of the phosphatase Cdc25 (which dephosphorylates and activates cdc2) during prophase I did not affect phosphorylation at the site recognized by either AD2 or TG3 at 0 or 6 h post-injection, while AT100 phosphorylation was not observed at either time point. Similar changes in tau phosphorylation were observed in stable tau-expressing neuroblastoma cells, indicating that the phosphorylation state of tau is modulated by mitotic events [40]. Given the results of these [40,41] and other studies [43,44], it has been suggested that in Alzheimer's disease brain neurons may be inappropriately trying to re-enter the cell cycle and this results in activation of mitotic kinases that can phosphorylate tau. It has also been speculated that because the neuron cannot re-enter the cell cycle properly, it subsequently defaults to a cell death pathway, which ultimately results in the death of the cell [45,46].

Xenopus oocytes have also been used as an in vivo assay to evaluate the effects of wild-type and FTDP-17 mutant tau on microtubule function [42]. Using human four-repeat tau, the authors demonstrated that wild-type tau, along with R406W and S305N mutants, inhibited oocyte maturation in a gene dose-dependent manner, presumably due to the fact that tau binds microtubule instability. In contrast, the P301L, ΔK280, G272V, P301S, V337M, three-repeat wild-type, and three-repeat R406W tau all had no effect on oocyte maturation, apparently because they may not bind the microtubules as efficiently as four-repeat tau and the R406W and S305N mutants [42]. These studies indicate that *Xenopus* oocytes may provide a unique, in vivo, environment to monitor tau phosphorylation and tau's effects on microtubules.

Although tau phosphorylation and function has not been extensively studied using non-mammalian systems as models, much useful information has already been obtained from the data that has been published. That is not to say that these models are not without their problems. For example, findings that have been reported using the different models are not always in agreement. Further, the data obtained using *Xenopus*, lamprey, *C. elegans*, or zebrafish as the model system need to be further substantiated by additional groups, as each model system has been used by only one lab to study tau phosphorylation and function. Despite these issues, the further development of non-mammalian model systems should vastly improve the knowledge base of tau function, phosphorylation, and dysfunction.

4. Tau phosphorylation in mammalian cell culture models

4.1. GSK3β and tau

Mammalian cell culture systems, both clonal and primary, have proved extremely useful in elucidating the signaling pathways that regulate tau phosphorylation and the effects of specific phosphorylation events on tau function. One protein kinase that has garnered significant attention as a "tau kinase" is GSK3β. Early studies showed that co-transfection of tau and GSK3β into nonneuronal cells resulted in a robust increase in tau phosphorylation at numerous epitopes. Further, co-expression of GSK3β with tau significantly impaired the binding of tau to microtubules (for review, see Ref. [29]). However, given the fact that GSK3β phosphorylates numerous sites on tau, especially in overexpression models, these studies did not allow for the evaluation of the effects of specific phospho-epitopes on tau function. This is an especially important question as GSK3β phosphorylates substrates at both unprimed Ser/Thr-Pro motifs and primed sites in which a Ser or Thr residue, four amino acids C-terminal to the target Ser/Thr, is phosphorylated first (for a review, see Ref. [24]), and there is increasing evidence that most physiologically relevant GSK3β substrates are primed [47,48]. In tau both unprimed and primed sites are phosphorylated by GSK3β, with Thr^{231} being the most noteworthy primed epitope [49–51]. Intriguingly, it was recently demonstrated in a co-transfection study that a GSK3β mutant that could only phosphorylate unprimed sites (GSK3β-R96A) [52,53] had no negative impact on tau's ability to bind microtubules, in comparison to wild-type GSK3β which significantly impaired tau's ability to bind microtubules [54]. Further, GSK3β-R96A phosphorylated unprimed sites on tau more efficiently than wild-type GSK3β, clearly indicating the importance of primed site phosphorylation in regulating tau-microtubule interactions [54]. Subsequent to this initial study it was demonstrated that primed phosphorylation of tau at Thr^{231} by GSK3β played a critical role in decreasing tau's ability to both bind and stabilize microtubules [55]. In transfected cells, tau with Thr^{231} mutated to an Ala was still able to efficiently bind microtubules after phosphorylation with GSK3β [55]. These studies clearly indicate that although GSK3β can phosphorylate numerous sites on tau, not all have an impact on tau function.

GSK3β may also play a role in regulating the degradation of tau. Tau is likely degraded by the proteasome [56], and proteasomal inhibition in concert with an enhancement of tau phosphorylation results in the aggregation of tau in oligodendroglial cells [57]. In a recent study, it was demonstrated that phosphorylation of tau by GSK3β resulted in binding by Hsc70 and recruitment of the E3 ligase CHIP which subsequently resulted in tau ubiquitination. Further, the presence of CHIP attenuated the cell death which occurred in response to overexpression of tau and GSK3β [58].

Given the likelihood that GSK3β plays a significant role in regulating tau phosphorylation in vivo, it is of critical importance to understanding the signaling pathways that modulate the activity of GSK3β. It is well established that phosphorylation of Ser^{9} on GSK3β inhibits its activity, while phosphorylation of Tyr^{216} increases activity (for a review, see Ref. [24]). Long-term treatment of cells with insulin or IGF-1 results in Akt activation, which in turn phosphorylates GSK3β on Ser^{9}, decreasing activity with the net result being a decrease in tau phosphorylation [59,60]. Interestingly, short-term exposure of clonal cells and primary neurons to insulin or IGF-1 results in an increase GSK3β-mediated tau phosphorylation, possibly due to activation of the tyrosine kinase fyn and GSK3β tyrosine phosphorylation [60,61]. In primary neurons, the transient activation of GSK3β by IGF-1 resulted in a relocation of tau to the distal portion of the axon [60]. There is also data demonstrating that integrin-linked kinase (ILK) phosphorylates GSK3β on Ser^{9}, inhibiting activity. In N1E-115 mouse neuroblastoma cells, inactivation of ILK resulted in an increase in tau phosphorylation that was ameliorated by treatment with LiCl, a GSK3 inhibitor [62]. In PC12 cells, similar findings were reported, with ILK inhibition resulting in increased tau phosphorylation and decreased neurite outgrowth in a GSK3-dependent manner [63].

Calcium has also been reported to regulate GSK3β activity. A modest, transient increase in intracellular calcium resulted in a prolonged increase in GSK3-dependent tau phosphorylation. Further, the increase in GSK3β-mediated tau phosphorylation was accompanied by an increase in the phosphorylation of Tyr^{216}, suggesting that the calcium transient resulted in activation of a calcium-dependent GSK3 phosphorylation [64]. However, recent studies from Phillip Cohen's lab have suggested that almost all the tyrosine phosphorylation of GSK3β is due to autophosphorylation [65], and therefore further studies are required to determine the mechanisms that are responsible for the increase GSK3β tyrosine phosphorylation in response to calcium transients.

Protein complexes that bind GSK3β play a significant role in regulating the activity and targets of GSK3β. The best characterized of these is the canonical wnt pathway. In the absence of a stimulus, the scaffold protein axin binds GSK3β, β-catenin, and other proteins that facilitate the phosphorylation and degradation of β-catenin. Wnt stimulation activates dishevelled (dvl), which, in concert with the GSK3 binding protein, frequently rearranged in advanced T cell lymphomas (FRAT), facilitates the dissociation of GSK3β from axin, thus decreasing β-catenin phosphorylation, resulting in β-catenin accumulation and activation (for a review, see Ref. [24]). Although the effects of this wnt signaling complex on GSK3β-mediated β-catenin phosphorylation are well established,

how this complex affects the phosphorylation of other GSK3β substrates, including tau, has not been fully elucidated. Overexpression of dvl attenuated the increase in GSK3β-mediated tau phosphorylation [66], although the mechanism of action is unclear as dvl does not bind GSKβ directly [67]. In addition, although overexpression of GSK3β did not increase phosphorylation at $Ser^{262/356}$ (12E8 epitope), which was expected as this epitope is not phosphorylated by GSK3β [68], dvl significantly decreased 12E8 immunoreactivity to below basal [66], suggesting that dvl may regulate tau phosphorylation through a GSK3β-independent pathway as well. Overexpression of FRAT appeared to attenuate GSK3β-mediated tau phosphorylation of $Ser^{396/404}$, and Ser residues between 189 and 206 [69]. Given the fact that FRAT directly interacts with GSK3β [67], these data suggest that this interaction may play a role in modulating GSK3β-mediated tau phosphorylation. Furthermore, the scaffolding protein axin potently inhibits tau phosphorylation by GSK3β, apparently by sequestering GSK3β away from tau and into the wnt signaling complex, as axin does not bind tau [70]. This suggests that the pool of GSK3β recruited by axin to phosphorylate β-catenin and modulate downstream signaling events is not readily available to phosphorylate tau, and perhaps other GSK3β substrates that do not interact with members of the wnt signaling complex.

In addition to regulating the interaction of tau with microtubules, GSK3β may play a role in the formation of insoluble tau fibrils. This would position GSK3β as a significant contributing factor in the development of tau pathology both in Alzheimer's disease and other neurodegenerative disorders. In COS-7 cells the expression of GSK3β with activated JNK3 resulted in significant tau phosphorylation and an increase in RIPA buffer insoluble tau aggregates [71]. Although intriguing, further studies are required to delineate the role of GSK3β in the formation of tau filaments in neurodegenerative diseases.

4.2. Cdk5 and tau

Cdk5 is a member of the cyclin-dependent kinase family, but is not involved in cell cycle regulation. The activity of this kinase is highest in neurons due to the selective expression of its regulator p35 in these cells. Cdk5/p35 plays a predominant role in brain development and function (for a review, see Ref. [72]). Cdk5, when complexed with p25 (a truncated form of p35), can phosphorylate tau at the epitopes that are similar to those that are phosphorylated during mitosis. Therefore, it has been suggested that Cdk5/p25 is responsible for the "mitotic-like" phosphorylation of tau in Alzheimer's disease brain [73]. There is data to suggest that because p25 is longer-lived than p35, Cdk5/p25 results in more extensive and maybe dysregulated substrate phosphorylation, compared to Cdk5/p35 (for a review, see Ref. [74]). Furthermore, p25 has been reported to be elevated in Alzheimer's disease brain [75,76], although these findings are controversial [77–79]. In addition, it should be noted that other kinases such as GSK3β can phosphorylate the same sites on tau as cdk5/p25 or cdc2/cyclin B1 (for a review, see Ref. [29]), and there are indications that GSK3β may also be dysregulated in Alzheimer's disease brain (for a review, see Ref. [24]). Therefore, although sites on tau that get phosphorylated during mitosis are also phosphorylated in Alzheimer's disease brain, whether or not cdk5, or other members of the cyclin dependent kinase family, directly contributes to the increase in tau phosphorylation remains unresolved.

4.3. Protein phosphatases and tau

In vitro, tau is readily dephosphorylated by numerous protein phosphatases, most notably PP1, PP2A and PP2B (for a review, see Ref. [80]). PP1 is targeted to microtubules by tau [81] and, recently, PP5 has been shown to dephosphorylate tau both in vitro and in situ when overexpressed in PC12 cells [82]. Nonetheless, the majority of studies have focused on the role of PP2A in regulating tau phosphorylation. The predominant brain isoform of PP2A, ABαC, binds directly to tau and is likely a major tau phosphatase [83,84]. It is also of interest to note that it has been hypothesized that Pin-1 regulates phosphorylation of Thr^{231} by selectively binding to this site and facilitating the conversion of the pThr-Pro motif to a trans conformation thus allowing dephosphorylation of the site by the predominant Pro-directed PP2A [51]. This is an interesting hypothesis; however, it should be noted that phosphorylation of peptides from this region of tau did not alter the equilibrium of cis-trans isomers [85]. There is also data to suggest that there is reduced binding of PP2A to tau with FTDP-17 mutations, which could contribute to the increase in tau phosphorylation that occurs in FTDP-17 cases [86].

4.4. Tyrosine phosphorylation of tau

In addition to being extensively phosphorylated on numerous Ser and Thr residues, tau is phosphorylated on Tyr^{18} [87]. The tyrosine kinase fyn binds to tau [88] and is likely the kinase that phosphorylates Tyr^{18}. Further, fyn can phosphorylate GSK3, which increases its activity resulting increased tau phosphorylation [61]. Therefore, the binding of fyn to tau may not only be responsible for the tyrosine phosphorylation of tau [61], but also may facilitate the phosphorylation of tau by GSK3β by enhancing the activity of this kinase. Treatment of cells with Aβ has been reported to result in a rapid and very transient increase in the tyrosine phosphorylation of cytoskeletal proteins, including tau, which is likely mediated by fyn [89]. Intriguingly, in oligodendrocytes the recruitment of tau and tubulin to fyn in lipid rafts

seems to be an important early step in the process of myelination [90]. Tyrosine phosphorylation of tau may also play a role in pathogenic processes, as PHF preparations stained with an antibody to phospho-Tyr[18], and in addition NFTs were also stained. Interestingly, neuropil threads and dystrophic neurites were not stained with the phospho-Tyr[18] antibody, suggesting a differential role of this phosphorylation event compared to the Ser/Thr phosphorylation in the pathogenic process [87].

4.5. The effects of cholesterol and Reelin on tau phosphorylation

Several epidemiological studies have found a correlation between elevated cholesterol levels and an increased risk of Alzheimer's disease (for a review, see Ref. [91]) and the use of statins (HMG-CoA reductase inhibitors) has been correlated with a decreased risk of dementia [92]. Further, tau hyperphosphorylation and tau pathology occur in Niemann–Pick Type C disease (NPC), which is a cholesterol storage disease with defects in intracellular trafficking of exogenous cholesterol [93]. Additionally, tau phosphorylation is increased in an NPC murine model [94]. These findings suggest that alterations in cholesterol metabolism may impact tau phosphorylation. However, in cultured neurons, treatment with an HMG-CoA reductase inhibitor, which decreases cholesterol biosynthesis, has been reported to result in increased tau phosphorylation [95]. In another study, inhibition of HMG-CoA reductase also resulted in increased tau phosphorylation in rat primary neuronal cultures, although the increases were modest and transient [96]. Although cholesterol depletion seems to enhance tau phosphorylation, treatment of primary hippocampal neurons with an HMG-CoA reductase inhibitor significantly decreased Aβ production [97,98]. Therefore, in terms of Alzheimer's disease pathology, the effects of HMG-CoA reductase inhibitors on tau phosphorylation and Aβ production appear paradoxical. These findings suggest that the decreased risk of dementia in patients who use statins may be due to the peripheral, not central, effects of the drugs, or that the predominant effect of the inhibitors in the brain may be to reduce Aβ production.

Members of the low-density lipoprotein (LDL) receptor gene family play important roles in the uptake and metabolism of lipids. Two members of this receptor family, the VLDL receptor and ApoER2, both bind ApoE and the extracellular matrix-associated protein Reelin (for a review, see Ref. [99]). Given that they are highly homologous to the LDL receptor, it was originally proposed that they played a role in lipoprotein metabolism; however, subsequent studies indicate that they have a significant signaling function and play crucial roles in neural development [99]. In terms of tau phosphorylation, it has been shown that knocking out the VLDL receptor and ApoER2, or Reelin, results in significant increases in tau phosphorylation [100]. This maybe due to the fact that Reelin activates phosphatidylinositol 3-kinase (PI3K) resulting in Akt activation and subsequently phosphorylation and inactivation of GSK3β. A likely outcome of this cascade of events would be a decrease in tau phosphorylation [101]. Given that tau mRNA is detectable in the rat from embryonic day 13/14 onwards [102], and therefore is present during Reelin-mediated neuronal migratory events that require significant alterations in the cytoskeleton [103], it is highly possible that Reelin-mediated alterations in tau phosphorylation may play a role cortical lamination.

4.6. Tau phosphorylation and neurite outgrowth

Early studies clearly demonstrated that tau plays a significant role in axonal outgrowth and the establishment of neuronal polarity [104,105]. Further, coordinated modulation of the phosphorylation of specific epitopes on tau appears to be required for appropriate neurite outgrowth. Expression of various tau constructs in insect Sf9 cells demonstrated that phosphorylation of $Ser^{262/356}$ in the microtubule binding domains is required for the development of cell processes, while phosphorylation of Ser/Thr-Pro motifs in the regions that flank the microtubule binding domains inhibits the expression of cell extensions [106]. Further, it appears that microtubule affinity-regulating kinase (MARK) likely plays a role in the phosphorylation of $Ser^{262/356}$ in tau, which decreases tau's affinity for microtubules and thus increases the dynamics of microtubules, which is required for the growth of neurites and the development of neuronal polarity [107]. Intriguingly, MARK was one of the proteins identified as a suppressor of tau toxicity in a genetic screen in *Drosophila* [25] (see above). In addition to MARK, GSK3β-mediated tau phosphorylation seems to be important in regulating neurite extension and retraction. Treatment of cells with lysophosphatidic acid results in activation of GSK3β, increased tau phosphorylation and neurite retraction [108,109]. Conversely, activation of ILK results in Akt activation and subsequent inhibitory phosphorylation of GSK3β and an attenuation of tau phosphorylation. This cascade of events plays a role in facilitating neurite outgrowth [63]. Taken together, all these findings support the hypothesis that increased phosphorylation of tau within the microtubule binding domains and a concomitant decrease in phosphorylation of tau in the flanking domains are required for appropriate neurite outgrowth.

4.7. Aβ-mediated tau phosphorylation

Alzheimer's disease is characterized by the presence of both extracellular amyloid plaques and intracellular NFTs, and there is increasing evidence that Aβ, which is the predominant component of the amyloid plaques, affects tau phosphorylation. Aβ fibrils induce tau phosphorylation

in rat hippocampal and human cortical neuronal cultures [110]. Mutations in the presenilin genes cause early onset Alzheimer's disease (for a review, see Ref. [111]), and presenilin 1 is one of the components of the γ-secretase complex, which is one of the proteases required for Aβ production [112]. In rat hippocampal neurons, expression of mutant presenilin 1 exacerbated Aβ-induced increases in tau phosphorylation, concomitant with increased neuritic dystrophy [113]. Treatment of tau-transfected human neuroblastoma SH-SY5Y cells with aggregated $A\beta_{42}$ for 5 days resulted in a decrease in the solubility of tau and the presence of filamentous tau. Phosphorylation of Ser^{422} appeared to play a role in this process, as mutation of Ser^{422} to Ala prevented tau aggregation and filament formation [114]. This finding is interesting for two reasons. First, there is data to suggest that phosphorylation of the C-terminal of tau significantly increases tau filament formation in vitro. Although in this previous study $Ser^{396/404}$ were pseudophosphorylated [115], a similar result might be expected for Ser^{422} phosphorylation. Second, caspase cleaves tau at Asp^{421}/Ser^{422} [116], and caspase-cleaved tau is very fibrillogenic [117] and is present in the NFTs in Alzheimer's disease brain [118]. Therefore, it can be suggested that phosphorylation of Ser^{422} of tau may enhance caspase cleavage and thus facilitate tau polymerization.

4.8. FTDP-17 tau phosphorylation

Numerous different mutations in the *tau* gene cause FTDP-17, and almost all neuropathologically examined FTDP-17 cases exhibit filamentous pathology containing hyperphosphorylated tau (for a review, see Ref. [119]). Although hyperphosphorylated tau is a characteristic of FTDP-17 cases, expression of FTDP-17 mutant tau in cell culture models has yielded variable results. In stably transfected CHO cells, most of the FTDP-17 tau mutants were phosphorylated to the same extent as wild-type tau, except for R406W tau, which showed a pronounced decrease in phosphorylation [120,121]. In transiently transfected CHO, all the FTDP-17 tau mutants (V337M, P301L and R406W) showed diminished PHF-1 immunoreactivity compared to wild-type tau, indicating that the mutants were phosphorylated significantly less than the wild-type tau at $Ser^{396/404}$ [122]. Transient transfection of human neuroblastoma SH-SY5Y cells with FTDP-17 mutant tau constructs yielded similar results, with the phosphorylation state of the mutant tau being equal to or less than the wild-type tau [123]. In another study, SH-SY5Y cells were engineered to inducibly express wild-type or FTDP-17 mutant tau. Although a 40% increase in phosphorylation at the PHF-1 site was noted for the V337M mutant tau compared to wild-type, phosphorylation at all other sites was significantly decreased, and for R406W mutant tau, phosphorylation was significantly decreased relative to wild-type tau for all epitopes examined [124]. Therefore, there has been some difficulty in recapitulating the increases in tau phosphorylation that occurs in FTDP-17 brains in cell culture model systems. However, in a recent study it was demonstrated that R406W tau stably overexpressed in immortalized mouse cortical cells was more highly phosphorylated than wild-type tau at numerous epitopes and showed decreased microtubule binding, an effect that could be reversed by dephosphorylation. These results suggest that expression of FTDP-17 mutant tau in the appropriate cellular context results in increases in phosphorylation that resemble the changes that occur in FTDP-17 brain [125].

4.9. "*Pseudophosphorylation*" *as an approach to examining phosphorylation effects on tau function*

Mutation of Ser/Thr residues to Glu or Asp residues has been used as an approach to mimic phosphorylation. For example, Akt is activated by phosphorylation of Thr^{308} and Ser^{473}, and conversion of these sites to Glu results in an Akt construct that is constitutively active (e.g., see Ref. [126]). Further, mutation of Ser^{396} and Ser^{404} to Glu was used to mimic the effects of phosphorylation of these sites on in vitro tau filament formation [115], and conversion of specific PKA phosphorylation sites on tau to Asp resulted in conformational changes similar to those observed in response to phosphorylation [127]. Pseudophosphorylated tau constructs may be useful for examining the effects of site-specific phosphorylation on tau function in cellular model systems. The primary approach has been to co-express tau with various protein kinases and determine functional outcomes. However, almost all protein kinases phosphorylate tau on more than one site, and additionally the protein kinases phosphorylate proteins other than tau. Therefore, it is often difficult to determine how phosphorylation of a particular site on tau directly impacts tau function. Recent studies have shown that expression of pseudophosphorylated tau results in functional impairment similar to tau that has been phosphorylated on those sites [128,129]. Therefore, the use of pseudophosphorylated tau constructs, in addition to studies using wild-type tau and specific protein kinases, may help provide more information on how the different phosphorylated epitopes on tau impact tau function.

5. Tau in transgenic mouse models

Over the past 10 years, numerous tau mouse models have been made, especially since the discovery of the tau mutations that cause FTDP-17. Although overexpression of tau often results in a phenotype, currently no model accurately recapitulates the cellular and nervous system pathology that occurs in the human diseases. Nonetheless, much has been learned about the phosphorylation, function

and dysfunction of tau from these models. Since tau transgenic mouse models have been discussed in a review published in 2002 [130], here we will just highlight several recent mouse models that provide new insights into the mechanisms that may contribute to aberrant tau phosphorylation and function in the disease state.

5.1. Tau and Cdk5/p35[p25] in transgenic models

In vitro cdk5 phosphorylates tau [131,132]. For cdk5 to be active, it must form a heterodimer with one of its regulatory subunits: p35, p25 (the truncated carboxy-terminus of p35), p39 or p29 (the truncated carboxy-terminus of p39) (for a review, see Ref. [74]). Given that these regulators are necessary for cdk5 activity, genetic manipulation of the activity of cdk5 is usually carried out by knocking out or overexpressing one of these regulatory proteins. Interestingly, in terms of tau phosphorylation and aggregation, these models have produced conflicting results. In one model of p25 overexpression, increased phosphorylation of tau was detected using immunohistochemical techniques, although the immunoblot data did not show a p25-induced increase in tau phosphorylation [133]. In another mouse model of p25 overexpression, there was widespread axonal degeneration; however, there was no evidence of NFTs. In addition, in this study no increase in tau phosphorylation was reported [134]. No significant differences in tau phosphorylation at the PHF-1 epitope (phospho-Ser^{396}/Ser^{404}) in cerebral cortex lysates from p35 knockout mice and p35 knockout mice expressing low levels of a p25 transgene were observed [135], and no increase in tau phosphorylation was observed in triple transgenic mice overexpressing p35, CDK5 and human tau [136]. Interestingly, mice expressing low levels of p25 in the postnatal forebrain showed increased levels of tau, but no increase in tau phosphorylation (although phosphorylation of NF-M was increased) [137]. It is also of interest that in p35 null mice where cdk5 activity is significantly decreased, the extent of tau phosphorylation is actually increased, which maybe due to the increase in GSK3β activity that was observed in these mice [138]. These findings suggest that cdk5 may not be directly phosphorylating tau in vivo. Nonetheless, given the number of substrates that are phosphorylated by cdk5, it is likely that cdk5 may indirectly modulate tau phosphorylation (for a review, see Ref. [74]).

In contrast to the above papers, two very recent reports show that p25 overexpression increases tau phosphorylation and pathology in mouse brain. Cruz et al. [139] demonstrated that overexpression of p25 in the postnatal mouse brain increased tau phosphorylation and the presence of tau in insoluble fractions. Along with these changes, affected brain regions showed increased neuronal dysfunction and loss and neurofibrillary pathology. Interestingly, based on their findings, these authors speculated that cdk5/p25 may induce neurodegeneration and increased tau phosphorylation and aggregation through independent parallel pathways [139]. In another recent study, the extent of tau phosphorylation at several epitopes in p25×P301L mutant tau double transgenic mice was found to be greater than that observed in the P301L tau mice. Further, the number of Gallyas silver-stained neurons in the brainstem of the p25×P301L mutant tau double transgenic mice was significantly greater than in P301L mice. Interestingly the levels of active GSK3β were increased in the double transgenic mice and some colocalization of cdk5, active GSK3β and aggregated tau was observed [140]. It is interesting to note that both knocking out p35 [138] and overexpressing p25 [140] led to increased GSK3β activity. Considering these findings, it is tempting to speculate that it is the dysregulation of GSK3β that directly leads to increased tau phosphorylation, rather than cdk5. However, further studies are clearly needed to determine the role of cdk5 in the tau pathogenic processes given the conflicting data that have been obtained from these different mouse models.

5.2. Tau and GSK3β in transgenic models

While much research has focused on GSK3β as a tau kinase both in vitro and in cell culture models, transgenic mouse studies employing GSK3β overexpression have been limited, presumably due to a lethal phenotype associated with GSK3β overexpression at high levels. Despite this fact, there have been several studies that have reported GSK3β overexpression in mouse models in a region-specific or inducible manner. Expression of a constitutively active GSK3β (S9A) in neonatal neurons led to a significant decrease in overall brain weight and volume, with the largest reduction occurring in the cerebral cortex [141]. Overexpression of GSK3β has also been shown to up- or down-regulate the expression of numerous proteins in neurons, including down-regulation of the 1 and β1 tubulin subunits [142].

Recent reports have shown that conditional overexpression of GSK3β leads to increased tau phosphorylation, decreased nuclear β-catenin, and increased neurodegeneration [143,144]. As with other models, the extent of GSK3β overexpression was modest, with an approximately 30% increase in activity. This modest increase in activity, however, translated into significant increases in tau phosphorylation at known GSK3β epitopes (e.g., AD-2/PHF-1), with no increase seen at the 12E8 epitope [143]. This is important, as it has been shown previously that GSK3β does not phosphorylate the 12E8 epitope [68]. Interestingly, despite the increases in tau phosphorylation on Alzheimer's disease-relevant epitopes, there was no evidence of tau aggregation, even though there was clear evidence of neurodegeneration [144].

In studies using tau/amyloid precursor protein (APP) transgenic mice, activated endogenous GSK3β has been shown to localize to degenerating neurons with filamentous tau pathology [145], suggesting that GSK3β activity may contribute to tau pathology. This is in contrast to previous

studies in human tau transgenic mice or culture neurons from tau transgenic mice, in which expression of GSK3β increased tau phosphorylation and alleviated tau-associated neurodegenerative changes [146,147]. In these studies, expression of GSK3β decreased tau-microtubule interactions and attenuated the axonal degeneration induced by tau overexpression, while not decreasing the amount of insoluble tau [146,147]. Treatment of transgenic mice that express tau with three FTDP-17 mutations with the GSK3 inhibitor LiCl resulted in a reduction of filamentous aggregates [148]. Although interesting, the phosphorylation levels of tau from treated and untreated animals were not clearly shown, so the reduction in filamentous aggregates cannot be attributed solely to an inhibition of tau phosphorylation by GSK3β, as LiCl treatment is neuroprotective independent of its effects on tau phosphorylation [149].

5.3. Tau and other Alzheimer's disease-relevant proteins in transgenic models

Since the identification of proteins which are either mutated in familial Alzheimer's disease cases (APP, presenilin-1/2) or show a strong genetic correlation with Alzheimer's disease (apoE4), numerous mouse models have been developed in which these proteins are either overexpressed or knocked out. These studies have yielded significant findings on how changes in these proteins may lead to cellular dysfunction and cell death (reviewed in Ref. [150]). Despite the accurate recapitulation of certain aspects of Alzheimer's disease in these models, there has typically been a lack of tau pathology and neuronal death in relevant brain areas. Recently, however, a number of groups have made transgenic models that express more than one Alzheimer's disease-related protein, and in some cases have been able to attain more Alzheimer's disease-like pathology, including tau pathology and neuronal death.

The presence of the ApoE4 allele is a risk factor for sporadic Alzheimer's disease. The ApoE4 protein itself, a 34-kDa protein of 299 amino acids, is found in Aβ plaques and associated with NFTs in Alzheimer's disease brain. The role of this protein in the pathogenesis of Alzheimer's disease is still unclear. Several recent studies have focused on the effects of ApoE4 on tau phosphorylation and function, and have yielded contrasting results. Mice that express knocked-in human ApoE4 show only a minimal increase in tau phosphorylation at Ser^{262} and Ser^{413}, and a modest decrease in Ser^{202}/Thr^{205} (AT8) and Thr^{205} phosphorylation [151]. Harris et al. [152] found that transgenic mice overexpressing C-terminal truncated human ApoE4 showed increased AT8 site phosphorylation. This group also reported the presence of "pre-tangle" inclusions and polymeric tau aggregates in these ApoE4 transgenic mice, which also exhibited neuronal degeneration in brain areas that expressed the transgene. These results are in agreement with cell culture studies which showed that ApoE4 increases tau phosphorylation and GSK3β activity, and even induces the formation of filamentous tau inclusions [153,154]. This has led some researchers to postulate that the C-terminal fragments of ApoE4 are sufficient to induce tau phosphorylation and aggregation [152,154]. However, the extent of tau phosphorylation in these models is relatively minor.

ApoE also seems to influence the activation of the Reelin pathway, which modulates GSK3β activity. The absence of ApoE in Reelin knockout mice increases GSK3β protein levels and activity, thereby increasing tau phosphorylation as well [155]. These authors suggest a model in which ApoE and Reelin function as ligands in a novel signaling pathway that works to suppress kinase activity toward tau. A model in which ApoE4 alone leads to increased kinase activity and tau phosphorylation changes was not proposed. Overall, the function of ApoE4 in normal and pathogenic brain processes remains unclear, and further studies are required.

Mouse models have been established that express either APP or mutant APP in combination with mutant presenilin-1, and these models have been used to examine the effects of these proteins on endogenous tau phosphorylation. Although in the majority of these studies there is increased tau phosphorylation, the particular outcomes vary [156–159]. One study reported PHF-like structures in brains of mice double transgenic for mutant APP and mutant presenilin-1 [158]. Other double transgenic APP/presenilin-1 mouse models established on the same, or similar, genetic background show increased neuritic pathology, Aβ deposition, and tau phosphorylation, however, without the aggregation of tau [156,157,159]. In these models the phosphorylation of endogenous tau is increased and often seems to localize to dystrophic neurites surrounding Aβ plaques, indicating a possible role of Aβ deposition in inducing aberrant tau phosphorylation. Presenilin-1 has also been shown to decrease GSK3β Ser^9 phosphorylation (which results in increased GSK3β activity) and disrupt kinesin-based microtubule transport [160]. This could partially account for the increased endogenous tau phosphorylation seen in mice that overexpress mutant presenilin-1 [160].

In mutant APP×P301L mutant tau double transgenic mice NFTs are present in the limbic areas [161]. Interestingly though, the NFTs were usually not in the vicinity of the amyloid plaques. Further, when Aβ fibrils are injected into P301L tau mice, tau phosphorylation and aggregation increased [162]. As in the APP×P301L mutant tau double transgenic mice, the formation of NFTs in response to injection of the Aβ fibrils did not occur at the site of injection, but rather at distal locations [162]. Both of these studies clearly indicate that Aβ can induce abnormal changes in tau phosphorylation and initiate events that result in increased tau aggregation. However, the mechanisms by which Aβ induces these changes in tau appear to be independent of the amyloid plaques per se.

To more accurately model all the pathological features of human Alzheimer's disease brain, triple transgenic mice have been made. These mice are transgenic for human tau (both wild-type and FTDP-17 mutant), mutant APP, and

mutant presenilin-1. Two separate groups have generated triple transgenic lines; however, there are differences. The triple transgenic mouse lines created by Oddo et al. [163] were generated by injecting the tau and APP genes into the embryos from mutant presenilin-1 knock-in mice, while Boutajangout et al. [164] established their triple transgenic mouse lines by selective breeding of three separate transgenic lines. Further, the transgenic mice described by Boutajangout et al. [164] express wild-type human tau and the S751L APP mutant, as well as mutant presenilin-1 (M146L), while triple transgenic mice described by Oddo et al. [163] express P301L mutant tau, the APP_{SWE} double mutant (K670M/N671L) and mutant presenilin-1 (M146V). Despite these differences between these two triple transgenic mouse lines, the results obtained from both lines show some similarities. In both lines, Aβ deposition was observed prior to the presence of noticeable tau accumulation. The tau accumulation was time-dependent and was also accompanied by increased phosphorylation at numerous epitopes as indicated by immunohistochemical staining [164]. It should be pointed out, however, that neither study demonstrated unequivocally that tau phosphorylation was increased in the triple transgenics over wild-type animals or single tau transgenic animals by immunoblotting, so the phosphorylation events that occur in both of these models needs to be further characterized. With that being said, the sequence of pathological events seems to be fairly consistent, both temporally and spatially. Tau aggregation was followed by neuritic changes, neuronal dysfunction, and eventually cell death. Interestingly, in the initial study [163], and in a follow-up study [165], Oddo and coworkers showed that tau pathology first appears in the hippocampus and then spreads to the cortex, while, conversely, Aβ deposition is first seen in the cortex and then subsequently in the hippocampus, implying a spatial disparity in the link between Aβ deposition and tau pathology. These results are similar to those observed in the mutant APP×P301L mutant tau double transgenic mice [161] and in the P301L mice injected with Aβ fibrils [162], in that the amyloid plaques and NFTs are not spatially related. Overall, these findings indicate that Aβ does facilitate the processes that lead to increased tau phosphorylation, but it is not the deposited plaques that are the causative factor. Rather, these data suggest that the diffusible forms of Aβ are likely important players in the cascade of events that leads to tau pathology and eventually neuronal dysfunction and death.

6. Conclusions

Over the past several years, substantial progress has been made in understanding the events that regulate tau phosphorylation and function. Further, there have been significant insights into the sequelae of events that contribute to the abnormal phosphorylation and accumulation of tau in Alzheimer's disease and other tau-related diseases. Although in Alzheimer's disease Aβ likely plays a role upstream of tau, aberrant tau phosphorylation and oligomerization are likely central to the disease process. Interestingly, the levels of tau phosphorylated at $Ser^{231/235}$ were significantly elevated in the CSF of patients with mild cognitive impairment (MCI) who later went on to develop Alzheimer's disease, compared to individuals who complained of memory problems but were without identifiable impairment by objective neuropsychological tests [166]. In another report, which used cases from the Religious Orders Study, NFTs and neuropil threads were significantly lower in persons without cognitive impairment compared to those with MCI and/or Alzheimer's disease. Further, the presence of NFTs was significantly correlated with decreases in episodic memory but not other cognitive abilities [167]. In a related study, Ghoshal et al. [168] showed that during the progression of Alzheimer's disease, neuropil threads precede the formation of PHFs, which in turn precede the appearance of tau-positive neuritic plaques. Further, increased staining with several of the tau antibodies correlated with deficits in episodic memory tasks. These and other findings (e.g., knocking out tau makes neurons resistant to Aβ induced toxicity [169]) strongly suggest that alterations in tau play a key role in the pathogenesis of Alzheimer's disease.

A model has been proposed [170] in which abnormal Aβ production and interactions occur upstream of tau dysfunction. Further, tau pathology can also be propagated by other pathways or by genetic alterations. In Fig. 1, a model is shown which is based on the one originally proposed by John Hardy in which numerous initiating events lead to changes in

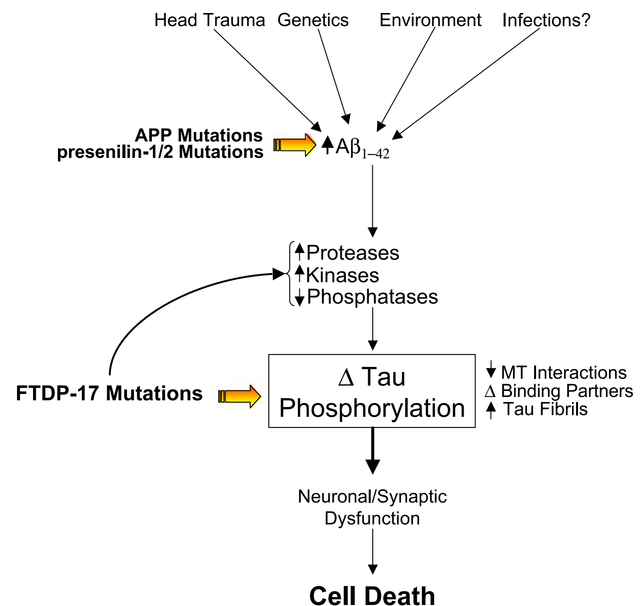

Fig. 1. Diagram illustrating events that may contribute to neuronal dysfunction in Alzheimer's disease and/or the FTDP-17 tauopathies and the pivotal role played by alterations in tau phosphorylation. This diagram is modified from a model originally proposed by Hardy [170].

tau phosphorylation, function, and localization (Fig. 1). Specifically, initiating events such as head trauma, genetic variables, infections, or other environmental factors lead to increased levels of $A\beta_{1-42}$ [171]. This could lead to events such as increased kinase and protease activity, and decreased phosphatase activity. The net result of these changes is sequential and specific increases in tau phosphorylation, which then leads to abnormal tau interactions and mislocalization, which are key events in the pathogenic processes that result in neuronal dysfunction and death. Fig. 2 illustrates the proposed stepwise changes in tau phosphorylation and function that may contribute to the demise of the cell. This figure has been adapted from a model that was previously proposed by Abraha et al. [115]. In normal physiological conditions there is a balance between the phosphorylation and dephosphorylation of tau that regulates tau's binding to microtubules and interactions with other specific binding partners (Fig. 2A). It can be hypothesized that "initiating" phosphorylation events in the tau pathogenic process would disrupt the normal localization of tau, presumably its association with microtubules (Fig. 2B). Once tau is displaced from microtubules, this would result in pathological phosphorylation at fibrillogenic sites and/or cleavage by proteases (i.e., caspases) resulting in a decrease in tau's binding to its normal partners and increased tau–tau interactions (which could be the key pathological event), and then subsequently tau filament formation and eventually aggregation into NFTs [115] (Fig. 2B). The mislocalization of tau due to abnormal phosphorylation and/or cleavage is likely to have numerous pathological ramifications. For example, these changes in tau could disrupt microtubule transport along the axons to the synapses, causing synaptic dysfunction. Synaptic dysfunction induced by aberrant posttranslational modifications of tau could result in cognitive deficits, which would then be exacerbated as affected neurons progress through dysfunction to death.

In conclusion, it is readily evident that abnormal changes in the phosphorylation state of tau play a key role in the pathogenesis of Alzheimer's disease and many other neurodegenerative diseases. What remains unclear are the

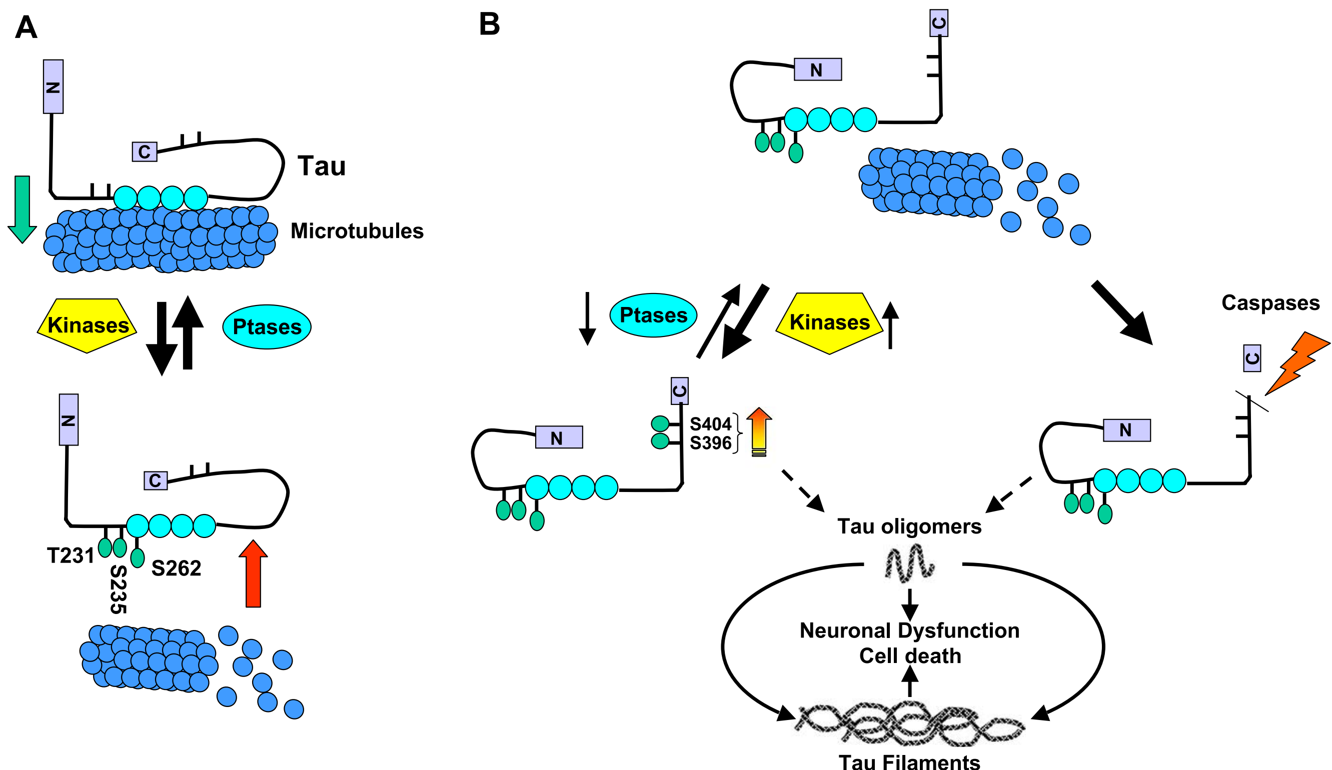

Fig. 2. Diagram illustrating the role of tau phosphorylation in regulating tau function in a physiological setting (A) or in a pathological situation (B). (A) Under physiological conditions, there are balanced and dynamic changes in tau phosphorylation, which modulate tau's interactions with microtubules allowing for appropriate neuronal function. Several different protein kinases and phosphatases likely work in concert to appropriately regulate tau phosphorylation. In particular, phosphorylation at Ser^{262} and Thr^{231}/Ser^{235} likely play key roles in regulating tau microtubule interactions. Normal kinase and phosphatase activities keep tau–microtubule interactions tightly regulated. (B) In a pathological state, certain toxic insults lead to a dysregulation in the balance in the activities of specific kinases and phosphatases, which results in tau being more phosphorylated at the critical microtubule regulatory sites leading to increased levels of "free tau" that is not bound to microtubules. This mislocalization of tau, along with the stressed state of the cell, could then result in further inappropriate phosphorylation events at fibrillogenic sites (e.g., $Ser^{396/404}$) and/or cleavage by caspases at Asp^{421}, which also increases the fibrillogenic properties of tau. These actions result in increased tau–tau interactions leading to the formation of oligomers and subsequently tau filaments. The decrease in tau's binding to microtubules as well as other physiological relevant proteins, coupled with an increase in self-association, contributes to the impairment of neuronal function and eventually cell death. This model was modified from one that was originally proposed by Abraha et al. [115].

signaling cascades and specific protein kinases and phosphatases that mediate these alterations in tau phosphorylation and function. What also remains to be elucidated are the specific functional changes in tau that are responsible for the pathological outcomes. Is it the phosphorylation-induced deficits in microtubule binding, the increased presence of tau fibrils, or other changes in tau localization and binding partners? Clearly, more work is needed to fully elucidate the role of site-specific phosphorylation in the normal functioning of tau and how these processes are perturbed and contribute to a pathogenic chain of events in the disease state.

References

[1] N. Shahani, R. Brandt, Functions and malfunctions of the tau proteins, Cell. Mol. Life Sci. 59 (2002) 1668–1680.

[2] M.D. Weingarten, A.H. Lockwood, S.Y. Hwo, M.W. Kirschner, A protein factor essential for microtubule assembly, Proc. Natl. Acad. Sci. U. S. A. 72 (1975) 1858–1862.

[3] D.W. Cleveland, S.Y. Hwo, M.W. Kirschner, Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly, J. Mol. Biol. 116 (1977) 227–247.

[4] G. Lindwall, R.D. Cole, Phosphorylation affects the ability of tau protein to promote microtubule assembly, J. Biol. Chem. 259 (1984) 5301–5305.

[5] K.S. Kosik, C.L. Joachim, D.J. Selkoe, Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 4044–4048.

[6] I. Grundke-Iqbal, K. Iqbal, Y.C. Tung, M. Quinlan, H.M. Wisniewski, L.I. Binder, Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 4913–4917.

[7] I. Grundke-Iqbal, K. Iqbal, M. Quinlan, Y.C. Tung, M.S. Zaidi, H.M. Wisniewski, Microtubule-associated protein tau. A component of Alzheimer paired helical filaments, J. Biol. Chem. 261 (1986) 6084–6089.

[8] R.E. Tanzi, J.F. Gusella, P.C. Watkins, G.A. Bruns, P. St. George-Hyslop, M.L. Van Keuren, D. Patterson, S. Pagan, D.M. Kurnit, R.L. Neve, Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus, Science 235 (1987) 880–884.

[9] P.H. St. George-Hyslop, R.E. Tanzi, R.J. Polinsky, J.L. Haines, L. Nee, P.C. Watkins, R.H. Myers, R.G. Feldman, D. Pollen, D. Drachman, J. Growdon, A. Bruni, J.-F. Foncin, D. Salmon, P. Frommelt, L. Amaducci, S. Sorbi, S. Piacentini, G.D. Stewart, W.J. Hobbs, P.M. Conneally, J.F. Gusella, The genetic defect causing familial Alzheimer's disease maps on chromosome 21, Science 235 (1987) 885–890.

[10] C.S. Atwood, M.E. Obrenovich, T. Liu, H. Chan, G. Perry, M.A. Smith, R.N. Martins, Amyloid-beta: a chameleon walking in two worlds: a review of the trophic and toxic properties of amyloid-beta, Brain Res. Brain Res. Rev. 43 (2003) 1–16.

[11] M. Baker, J.B. Kwok, S. Kucera, R. Crook, M. Farrer, H. Houlden, A. Isaacs, S. Lincoln, L. Onstead, J. Hardy, L. Wittenberg, P. Dodd, S. Webb, N. Hayward, T. Tannenberg, A. Andreadis, M. Hallupp, P. Schofield, F. Dark, M. Hutton, Localization of frontotemporal dementia with parkinsonism in an Australian kindred to chromosome 17q21–22, Ann. Neurol. 42 (1997) 794–798.

[12] M. Hutton, C.L. Lendon, P. Rizzu, M. Baker, S. Froelich, H. Houlden, S. Pickering-Brown, S. Chakraverty, A. Isaacs, A. Grover, J. Hackett, J. Adamson, S. Lincoln, D. Dickson, P. Davies, R.C. Petersen, M. Stevens, E. de Graaff, E. Wauters, J. van Baren, M. Hillebrand, M. Joosse, J.M. Kwon, P. Nowotny, L.K. Che, J. Norton, J.C. Morris, L.A. Reed, J. Trojanowski, H. Basun, L. Lannfelt, M.L. Neystat, S. Fahn, F. Dark, T. Tanneberg, P.R. Dodd, N. Hayward, J.B. Kwok, P.R. Schofield, A. Andreadis, J. Snowden, D. Craufurd, D. Neary, F. Owen, B.A. Oostra, J. Hardy, A. Goate, J. VanSwieten, D. Mann, T. Lynch, P. Heutink, Association of missense and 5′ -splice-site mutations in tau with the inherited dementia FTDP-17, Nature 393 (1998) 702–705.

[13] M.G. Spillantini, J.R. Murrell, M. Goedert, M.R. Farlow, A. Klug, B. Ghetti, Mutation in the tau gene in familial multiple system tauopathy with presenile dementia, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 7737–7741.

[14] P. Poorkaj, T.D. Bird, E. Wijsman, E. Nemens, R.M. Garruto, L. Anderson, A. Andreadis, W.C. Wiederholt, M. Raskind, G.D. Schellenberg, Tau is a candidate gene for chromosome 17 frontotemporal dementia, Ann. Neurol. 43 (1998) 815–825.

[15] D. Yancopoulou, M.G. Spillantini, Tau protein in familial and sporadic diseases, Neuromolecular Med. 4 (2003) 37–48.

[16] C. Gally, J.L. Bessereau, [C. elegans: of neurons and genes], Med. Sci. (Paris) 19 (2003) 725–734.

[17] B.C. Kraemer, B. Zhang, J.B. Leverenz, J.H. Thomas, J.Q. Trojanowski, G.D. Schellenberg, Neurodegeneration and defective neurotransmission in a *Caenorhabditis elegans* model of tauopathy, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9980–9985.

[18] H. Schmidt, C. Rickert, T. Bossing, O. Vef, J. Urban, G.M. Technau, The embryonic central nervous system lineages of *Drosophila melanogaster*: II. Neuroblast lineages derived from the dorsal part of the neuroectoderm, Dev. Biol. 189 (1997) 186–204.

[19] T. Bossing, G. Udolph, C.Q. Doe, G.M. Technau, The embryonic central nervous system lineages of *Drosophila melanogaster*: I. Neuroblast lineages derived from the ventral half of the neuroectoderm, Dev. Biol. 179 (1996) 41–64.

[20] V. Cambiazo, M. Gonzalez, R.B. Maccioni, DMAP-85: a tau-like protein from *Drosophila melanogaster* larvae, J. Neurochem. 64 (1995) 1288–1297.

[21] C.W. Wittmann, M.F. Wszolek, J.M. Shulman, P.M. Salvaterra, J. Lewis, M. Hutton, M.B. Feany, Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles, Science 293 (2001) 711–714.

[22] D.W. Williams, M. Tyrer, D. Shepherd, Tau and tau reporters disrupt central projections of sensory neurons in Drosophila, J. Comp. Neurol. 428 (2000) 630–640.

[23] G.R. Jackson, M. Wiedau-Pazos, T.K. Sang, N. Wagle, C.A. Brown, S. Massachi, D.H. Geschwind, Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila, Neuron 34 (2002) 509–519.

[24] R.S. Jope, G.V. Johnson, The glamour and gloom of glycogen synthase kinase 3 (GSK3), Trends Biol. Sci. 29 (2004) 95–102.

[25] J.M. Shulman, M.B. Feany, Genetic modifiers of tauopathy in Drosophila, Genetics 165 (2003) 1233–1242.

[26] B.H. Anderton, J. Betts, W.P. Blackstock, J.P. Brion, S. Chapman, J. Connell, R. Dayanandan, J.M. Gallo, G. Gibb, D.P. Hanger, M. Hutton, E. Kardalinou, K. Leroy, S. Lovestone, T. Mack, C.H. Reynolds, M. Van Slegtenhorst, Sites of phosphorylation in tau and factors affecting their regulation, Biochem. Soc. Symp. (2001) 73–80.

[27] J. Wang, X. Wang, R. Liu, Q. Wang, I. Grundke-Iqbal, K. Iqbal, In vitro analysis of tau phosphorylation sites and its biological activity, Chin. Med. Sci. J. 17 (2002) 13–16.

[28] H.Y. Wang, W. Li, N.J. Benedetti, D.H. Lee, Alpha 7 nicotinic acetylcholine receptors mediate beta-amyloid peptide-induced tau protein phosphorylation, J. Biol. Chem. 278 (2003) 31547–31553.

[29] G.V. Johnson, J.A. Hartigan, Tau protein in normal and Alzheimer's disease brain: an update, J. Alzheimer's Dis. 1 (1999) 329–351.

[30] D.H. Geschwind, Tau phosphorylation, tangles, and neurodegeneration: the chicken or the egg? Neuron 40 (2003) 457–460.

[31] T.G. Deliagina, P.V. Zelenin, G.N. Orlovsky, Encoding and decoding of reticulospinal commands, Brain Res. Brain Res. Rev. 40 (2002) 166–177.
[32] G.F. Hall, J. Yao, G. Lee, Human tau becomes phosphorylated and forms filamentous deposits when overexpressed in lamprey central neurons in situ, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 4733–4738.
[33] G.F. Hall, V.M. Lee, G. Lee, J. Yao, Staging of neurofibrillary degeneration caused by human tau overexpression in a unique cellular model of human tauopathy, Am. J. Pathol. 158 (2001) 235–246.
[34] G.F. Hall, PHF-tau from Alzheimer brain is rapidly dephosphorylated and degraded when injected into neurons in situ, J. Alzheimer's Dis. 1 (1999) 379–386.
[35] G.F. Hall, S. Lee, J. Yao, Neurofibrillary degeneration can be arrested in an in vivo cellular model of human tauopathy by application of a compound which inhibits tau filament formation in vitro, J. Mol. Neurosci. 19 (2002) 253–260.
[36] J.T. Shin, M.C. Fishman, From Zebrafish to human: modular medical models, Annu. Rev. Genomics Hum. Genet. 3 (2002) 311–340.
[37] A.C. Ward, G.J. Lieschke, The zebrafish as a model system for human disease, Front. Biosci. 7 (2002) d827–d833.
[38] G.E. Ackermann, B.H. Paw, Zebrafish: a genetic model for vertebrate organogenesis and human disorders, Front. Biosci. 8 (2003) d1227–d1253.
[39] H.G. Tomasiewicz, D.B. Flaherty, J.P. Soria, J.G. Wood, Transgenic zebrafish model of neurodegeneration, J. Neurosci. Res. 70 (2002) 734–745.
[40] P. Delobel, S. Flament, M. Hamdane, C. Mailliot, A.V. Sambo, S. Begard, N. Sergeant, A. Delacourte, J.P. Vilain, L. Buee, Abnormal tau phosphorylation of the Alzheimer-type also occurs during mitosis, J. Neurochem. 83 (2002) 412–420.
[41] P. Delobel, S. Flament, M. Hamdane, A. Delacourte, J.P. Vilain, L. Buee, Modelling Alzheimer-specific abnormal tau phosphorylation independently of GSK3beta and PKA kinase activities, FEBS Lett. 516 (2002) 151–155.
[42] P. Delobel, S. Flament, M. Hamdane, R. Jakes, A. Rousseau, A. Delacourte, J.P. Vilain, M. Goedert, L. Buee, Functional characterization of FTDP-17 tau gene mutations through their effects on Xenopus oocyte maturation, J. Biol. Chem. 277 (2002) 9199–9205.
[43] Y. Yang, D.S. Geldmacher, K. Herrup, DNA replication precedes neuronal cell death in Alzheimer's disease, J. Neurosci. 21 (2001) 2661–2668.
[44] Y. Yang, E.J. Mufson, K. Herrup, Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease, J. Neurosci. 23 (2003) 2557–2563.
[45] S.H. Lin, Z.Z. Chong, K. Maiese, Cell cycle induction in post-mitotic neurons proceeds in concert with the initial phase of programmed cell death in rat, Neurosci. Lett. 310 (2001) 173–177.
[46] R.S. Freeman, S. Estus, E.M. Johnson Jr., Analysis of cell cycle-related gene expression in postmitotic neurons: selective induction of Cyclin D1 during programmed cell death, Neuron 12 (1994) 343–355.
[47] C. Liu, Y. Li, M. Semenov, C. Han, G.H. Baeg, Y. Tan, Z. Zhang, X. Lin, X. He, Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism, Cell 108 (2002) 837–847.
[48] A.J. Harwood, Regulation of GSK-3: a cellular multiprocessor, Cell 105 (2001) 821–824.
[49] P.J. Lu, G. Wulf, X.Z. Zhou, P. Davies, K.P. Lu, The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein, Nature 399 (1999) 784–788.
[50] I. Vincent, G. Jicha, M. Rosado, D.W. Dickson, Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain, J. Neurosci. 17 (1997) 3588–3598.
[51] X.Z. Zhou, O. Kops, A. Werner, P.J. Lu, M. Shen, G. Stoller, G. Kullertz, M. Stark, G. Fischer, K.P. Lu, Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins, Mol. Cell 6 (2000) 873–883.
[52] S. Frame, P. Cohen, R.M. Biondi, A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation, Mol. Cell 7 (2001) 1321–1327.
[53] R. Dajani, E. Fraser, S.M. Roe, N. Young, V. Good, T.C. Dale, L.H. Pearl, Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition, Cell 105 (2001) 721–732.
[54] J.H. Cho, G.V. Johnson, Glycogen synthase kinase 3beta phosphorylates tau at both primed and unprimed sites. Differential impact on microtubule binding, J. Biol. Chem. 278 (2003) 187–193.
[55] J.H. Cho, G.V. Johnson, Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3beta (GSK3beta) plays a critical role in regulating tau's ability to bind and stabilize microtubules, J. Neurochem. 88 (2004) 349–358.
[56] D.C. David, R. Layfield, L. Serpell, Y. Narain, M. Goedert, M.G. Spillantini, Proteasomal degradation of tau protein, J. Neurochem. 83 (2002) 176–185.
[57] O. Goldbaum, M. Oppermann, M. Handschuh, D. Dabir, B. Zhang, M.S. Forman, J.Q. Trojanowski, V.M. Lee, C. Richter-Landsberg, Proteasome inhibition stabilizes tau inclusions in oligodendroglial cells that occur after treatment with okadaic acid, J. Neurosci. 23 (2003) 8872–8880.
[58] H. Shimura, D. Schwartz, S.P. Gygi, K.S. Kosik, CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival, J. Biol. Chem. 279 (2004) 4869–4876.
[59] M. Hong, V.M. Lee, Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons, J. Biol. Chem. 272 (1997) 19547–19553.
[60] M. Lesort, G.V. Johnson, Insulin-like growth factor-1 and insulin mediate transient site-selective increases in tau phosphorylation in primary cortical neurons, Neuroscience 99 (2000) 305–316.
[61] M. Lesort, R.S. Jope, G.V. Johnson, Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase, J. Neurochem. 72 (1999) 576–584.
[62] T. Ishii, H. Furuoka, Y. Muroi, M. Nishimura, Inactivation of integrin-linked kinase induces aberrant tau phosphorylation via sustained activation of glycogen synthase kinase 3beta in N1E-115 neuroblastoma cells, J. Biol. Chem. 278 (2003) 26970–26975.
[63] J. Mills, M. Digicaylioglu, A.T. Legg, C.E. Young, S.S. Young, A.M. Barr, L. Fletcher, T.P. O'Connor, S. Dedhar, Role of integrin-linked kinase in nerve growth factor-stimulated neurite outgrowth, J. Neurosci. 23 (2003) 1638–1648.
[64] J.A. Hartigan, G.V. Johnson, Transient increases in intracellular calcium result in prolonged site-selective increases in tau phosphorylation through a glycogen synthase kinase 3beta-dependent pathway, J. Biol. Chem. 274 (1999) 21395–21401.
[65] A. Cole, S. Frame, P. Cohen, Further evidence that the tyrosine phosphorylation of glycogen synthase kinase-3 (GSK3) in mammalian cells is an autophosphorylation event, Biochem. J. 377 (2004) 249–255.
[66] A. Mudher, S. Chapman, J. Richardson, A. Asuni, G. Gibb, C. Pollard, R. Killick, T. Iqbal, L. Raymond, I. Varndell, P. Sheppard, A. Makoff, E. Gower, P.E. Soden, P. Lewis, M. Murphy, T.E. Golde, H.T. Rupniak, B.H. Anderton, S. Lovestone, Dishevelled regulates the metabolism of amyloid precursor protein via protein kinase C/mitogen-activated protein kinase and c-Jun terminal kinase, J. Neurosci. 21 (2001) 4987–4995.
[67] L. Li, H. Yuan, C.D. Weaver, J. Mao, G.H. Farr III, D.J. Sussman, J. Jonkers, D. Kimelman, D. Wu, Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1, EMBO J. 18 (1999) 4233–4240.

[68] R. Godemann, J. Biernat, E. Mandelkow, E.M. Mandelkow, Phosphorylation of tau protein by recombinant GSK-3beta: pronounced phosphorylation at select Ser/Thr-Pro motifs but no phosphorylation at Ser262 in the repeat domain, FEBS Lett. 454 (1999) 157–164.
[69] A.A. Culbert, M.J. Brown, S. Frame, T. Hagen, D.A. Cross, B. Bax, A.D. Reith, GSK-3 inhibition by adenoviral FRAT1 overexpression is neuroprotective and induces tau dephosphorylation and beta-catenin stabilisation without elevation of glycogen synthase activity, FEBS Lett. 507 (2001) 288–294.
[70] W.H. Stoothoff, C.D. Bailey, K. Mi, S.C. Lin, G.V. Johnson, Axin negatively affects tau phosphorylation by glycogen synthase kinase 3beta, J. Neurochem. 83 (2002) 904–913.
[71] S. Sato, Y. Tatebayashi, T. Akagi, D.H. Chui, M. Murayama, T. Miyasaka, E. Planel, K. Tanemura, X. Sun, T. Hashikawa, K. Yoshioka, K. Ishiguro, A. Takashima, Aberrant tau phosphorylation by glycogen synthase kinase-3beta and JNK3 induces oligomeric tau fibrils in COS-7 cells, J. Biol. Chem. 277 (2002) 42060–42065.
[72] D.S. Smith, L.H. Tsai, Cdk5 behind the wheel: a role in trafficking and transport? Trends Cell Biol. 12 (2002) 28–36.
[73] M. Hamdane, A.V. Sambo, P. Delobel, S. Begard, A. Violleau, A. Delacourte, P. Bertrand, J. Benavides, L. Buee, Mitotic-like tau phosphorylation by p25-Cdk5 kinase complex, J. Biol. Chem. 278 (2003) 34026–34034.
[74] S.B. Shelton, G.V. Johnson, Cyclin-dependent kinase-5 in neurodegeneration, J. Neurochem. 88 (2004) 1313–1326.
[75] G.N. Patrick, L. Zukerberg, M. Nikolic, S. de la Monte, P. Dikkes, L.H. Tsai, Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration, Nature 402 (1999) 615–622.
[76] M.S. Lee, Y.T. Kwon, M. Li, J. Peng, R.M. Friedlander, L.H. Tsai, Neurotoxicity induces cleavage of p35 to p25 by calpain, Nature 405 (2000) 360–364.
[77] K.C. Nguyen, J.L. Rosales, M. Barboza, K.Y. Lee, Controversies over p25 in Alzheimer's disease, J. Alzheimer's Dis. 4 (2002) 123–126.
[78] A. Tandon, H. Yu, L. Wang, E. Rogaeva, C. Sato, M.A. Chishti, T. Kawarai, H. Hasegawa, F. Chen, P. Davies, P.E. Fraser, D. Westaway, P.H. St. George-Hyslop, Brain levels of CDK5 activator p25 are not increased in Alzheimer's or other neurodegenerative diseases with neurofibrillary tangles, J. Neurochem. 86 (2003) 572–581.
[79] B.C. Yoo, G. Lubec, p25 protein in neurodegeneration, Nature 411 (2001) 763–764 (discussion 764–765).
[80] Q. Tian, J. Wang, Role of serine/threonine protein phosphatase in Alzheimer's disease, NeuroSignals 11 (2002) 262–269.
[81] H. Liao, Y. Li, D.L. Brautigan, G.G. Gundersen, Protein phosphatase 1 is targeted to microtubules by the microtubule-associated protein Tau, J. Biol. Chem. 273 (1998) 21901–21908.
[82] C.X. Gong, F. Liu, G. Wu, S. Rossie, J. Wegiel, L. Li, I. Grundke-Iqbal, K. Iqbal, Dephosphorylation of microtubule-associated protein tau by protein phosphatase 5, J. Neurochem. 88 (2004) 298–310.
[83] E. Sontag, V. Nunbhakdi-Craig, G. Lee, G.S. Bloom, M.C. Mumby, Regulation of the phosphorylation state and microtubule-binding activity of tau by protein phosphatase 2A, Neuron 17 (1996) 1201–1207.
[84] E. Sontag, V. Nunbhakdi-Craig, G. Lee, R. Brandt, C. Kamibayashi, J. Kuret, C.L. White III, M.C. Mumby, G.S. Bloom, Molecular interactions among protein phosphatase 2A, tau, and microtubules. Implications for the regulation of tau phosphorylation and the development of tauopathies, J. Biol. Chem. 274 (1999) 25490–25498.
[85] N.L. Daly, R. Hoffmann, L. Otvos Jr., D.J. Craik, Role of phosphorylation in the conformation of tau peptides implicated in Alzheimer's disease, Biochemistry 39 (2000) 9039–9046.
[86] M. Goedert, S. Satumtira, R. Jakes, M.J. Smith, C. Kamibayashi, C.L. White 3rd, E. Sontag, Reduced binding of protein phosphatase 2A to tau protein with frontotemporal dementia and parkinsonism linked to chromosome 17 mutations, J. Neurochem. 75 (2000) 2155–2162.
[87] G. Lee, R. Thangavel, V.M. Sharma, J.M. Litersky, K. Bhaskar, S.M. Fang, L.H. Do, A. Andreadis, G. Van Hoesen, H. Ksiezak-Reding, Phosphorylation of tau by fyn, implications for Alzheimer's diseas, J. Neurosci. 24 (2004) 2304–2312.
[88] G. Lee, S.T. Newman, D.L. Gard, H. Band, G. Panchamoorthy, Tau interacts with src-family non-receptor tyrosine kinases, J. Cell Sci. 111 (Pt. 21) (1998) 3167–3177.
[89] R. Williamson, T. Scales, B.R. Clark, G. Gibb, C.H. Reynolds, S. Kellie, I.N. Bird, I.M. Varndell, P.W. Sheppard, I. Everall, B.H. Anderton, Rapid tyrosine phosphorylation of neuronal proteins including tau and focal adhesion kinase in response to amyloid-beta peptide exposure: involvement of Src family protein kinases, J. Neurosci. 22 (2002) 10–20.
[90] C. Klein, E.M. Kramer, A.M. Cardine, B. Schraven, R. Brandt, J. Trotter, Process outgrowth of oligodendrocytes is promoted by interaction of fyn kinase with the cytoskeletal protein tau, J. Neurosci. 22 (2002) 698–707.
[91] M. Burns, K. Duff, Cholesterol in Alzheimer's disease and tauopathy, Ann. N. Y. Acad. Sci. 977 (2002) 367–375.
[92] H. Jick, G.L. Zornberg, S.S. Jick, S. Seshadri, D.A. Drachman, Statins and the risk of dementia, Lancet 356 (2000) 1627–1631.
[93] I.A. Auer, M.L. Schmidt, V.M. Lee, B. Curry, K. Suzuki, R.W. Shin, P.G. Pentchev, E.D. Carstea, J.Q. Trojanowski, Paired helical filament tau (PHFtau) in Niemann–Pick type C disease is similar to PHFtau in Alzheimer's disease, Acta Neuropathol. (Berl.) 90 (1995) 547–551.
[94] B. Bu, J. Li, P. Davies, I. Vincent, Deregulation of cdk5, hyperphosphorylation, and cytoskeletal pathology in the Niemann–Pick type C murine model, J. Neurosci. 22 (2002) 6515–6525.
[95] Q.W. Fan, W. Yu, T. Senda, K. Yanagisawa, M. Michikawa, Cholesterol-dependent modulation of tau phosphorylation in cultured neurons, J. Neurochem. 76 (2001) 391–400.
[96] V. Meske, F. Albert, D. Richter, J. Schwarze, T.G. Ohm, Blockade of HMG-CoA reductase activity causes changes in microtubule-stabilizing protein tau via suppression of geranylgeranylpyrophosphate formation: implications for Alzheimer's disease, Eur. J. Neurosci. 17 (2003) 93–102.
[97] K. Fassbender, M. Simons, C. Bergmann, M. Stroick, D. Lutjohann, P. Keller, H. Runz, S. Kuhl, T. Bertsch, K. von Bergmann, M. Hennerici, K. Beyreuther, T. Hartmann, Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5856–5861.
[98] M. Simons, P. Keller, B. De Strooper, K. Beyreuther, C.G. Dotti, K. Simons, Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6460–6464.
[99] J. Herz, The LDL receptor gene family: (un)expected signal transducers in the brain, Neuron 29 (2001) 571–581.
[100] T. Hiesberger, M. Trommsdorff, B.W. Howell, A. Goffinet, M.C. Mumby, J.A. Cooper, J. Herz, Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation, Neuron 24 (1999) 481–489.
[101] U. Beffert, G. Morfini, H.H. Bock, H. Reyna, S.T. Brady, J. Herz, Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3beta, J. Biol. Chem. 277 (2002) 49958–49964.
[102] K.S. Kosik, L.D. Orecchio, S. Bakalis, R.L. Neve, Developmentally regulated expression of specific tau sequences, Neuron 2 (1989) 1389–1397.

[103] C.A. Walsh, A.M. Goffinet, Potential mechanisms of mutations that affect neuronal migration in man and mouse, Curr. Opin. Genet. Dev. 10 (2000) 270–274.
[104] A. Caceres, K.S. Kosik, Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons, Nature 343 (1990) 461–463.
[105] A. Caceres, S. Potrebic, K.S. Kosik, The effect of tau antisense oligonucleotides on neurite formation of cultured cerebellar macroneurons, J. Neurosci. 11 (1991) 1515–1523.
[106] J. Biernat, E.M. Mandelkow, The development of cell processes induced by tau protein requires phosphorylation of serine 262 and 356 in the repeat domain and is inhibited by phosphorylation in the proline-rich domains, Mol. Biol. Cell 10 (1999) 727–740.
[107] J. Biernat, Y.Z. Wu, T. Timm, Q. Zheng-Fischhofer, E. Mandelkow, L. Meijer, E.M. Mandelkow, Protein kinase MARK/PAR-1 is required for neurite outgrowth and establishment of neuronal polarity, Mol. Biol. Cell 13 (2002) 4013–4028.
[108] C.L. Sayas, J. Avila, F. Wandosell, Glycogen synthase kinase-3 is activated in neuronal cells by Galpha12 and Galpha13 by Rho-independent and Rho-dependent mechanisms, J. Neurosci. 22 (2002) 6863–6875.
[109] C.L. Sayas, J. Avila, F. Wandosell, Regulation of neuronal cytoskeleton by lysophosphatidic acid: role of GSK-3, Biochim. Biophys. Acta 1582 (2002) 144–153.
[110] J. Busciglio, A. Lorenzo, J. Yeh, B.A. Yankner, beta-Amyloid fibrils induce tau phosphorylation and loss of microtubule binding, Neuron 14 (1995) 879–888.
[111] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2001) 741–766.
[112] B. De Strooper, Aph-1, Pen-2, and nicastrin with presenilin generate an active gamma-secretase complex, Neuron 38 (2003) 9–12.
[113] G. Pigino, A. Pelsman, H. Mori, J. Busciglio, Presenilin-1 mutations reduce cytoskeletal association, deregulate neurite growth, and potentiate neuronal dystrophy and tau phosphorylation, J. Neurosci. 21 (2001) 834–842.
[114] A. Ferrari, F. Hoerndli, T. Baechi, R.M. Nitsch, J. Gotz, beta-Amyloid induces paired helical filament-like tau filaments in tissue culture, J. Biol. Chem. 278 (2003) 40162–40168.
[115] A. Abraha, N. Ghoshal, T.C. Gamblin, V. Cryns, R.W. Berry, J. Kuret, L.I. Binder, C-terminal inhibition of tau assembly in vitro and in Alzheimer's disease, J. Cell Sci. 113 (Pt. 21) (2000) 3737–3745.
[116] L. Fasulo, G. Ugolini, M. Visintin, A. Bradbury, C. Brancolini, V. Verzillo, M. Novak, A. Cattaneo, The neuronal microtubule-associated protein tau is a substrate for caspase-3 and an effector of apoptosis, J. Neurochem. 75 (2000) 624–633.
[117] R.W. Berry, A. Abraha, S. Lagalwar, N. LaPointe, T.C. Gamblin, V.L. Cryns, L.I. Binder, Inhibition of tau polymerization by its carboxy-terminal caspase cleavage fragment, Biochemistry 42 (2003) 8325–8331.
[118] T.C. Gamblin, F. Chen, A. Zambrano, A. Abraha, S. Lagalwar, A.L. Guillozet, M. Lu, Y. Fu, F. Garcia-Sierra, N. LaPointe, R. Miller, R.W. Berry, L.I. Binder, V.L. Cryns, Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10032–10037.
[119] E.M. Ingram, M.G. Spillantini, Tau gene mutations: dissecting the pathogenesis of FTDP-17, Trends Mol. Med. 8 (2002) 555–562.
[120] N. Matsumura, T. Yamazaki, Y. Ihara, Stable expression in Chinese hamster ovary cells of mutated tau genes causing frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), Am. J. Pathol. 154 (1999) 1649–1656.
[121] V. Vogelsberg-Ragaglia, J. Bruce, C. Richter-Landsberg, B. Zhang, M. Hong, J.Q. Trojanowski, V.M. Lee, Distinct FTDP-17 missense mutations in tau produce tau aggregates and other pathological phenotypes in transfected CHO cells, Mol. Biol. Cell 11 (2000) 4093–4104.
[122] R. Dayanandan, M. Van Slegtenhorst, T.G. Mack, L. Ko, S.H. Yen, K. Leroy, J.P. Brion, B.H. Anderton, M. Hutton, S. Lovestone, Mutations in tau reduce its microtubule binding properties in intact cells and affect its phosphorylation, FEBS Lett. 446 (1999) 228–232.
[123] T.G. Mack, R. Dayanandan, M. Van Slegtenhorst, A. Whone, M. Hutton, S. Lovestone, B.H. Anderton, Tau proteins with frontotemporal dementia-17 mutations have both altered expression levels and phosphorylation profiles in differentiated neuroblastoma cells, Neuroscience 108 (2001) 701–712.
[124] M. DeTure, L.W. Ko, C. Easson, S.H. Yen, Tau assembly in inducible transfectants expressing wild-type or FTDP-17 tau, Am. J. Pathol. 161 (2002) 1711–1722.
[125] P.K. Krishnamurthy, G.V. Johnson, Mutant (R406W) human tau is hyperphosphorylated and does not efficiently bind microtubules in a neuronal cortical cell model, J. Biol. Chem. (2003) 7893–7900.
[126] J. Liang, J. Zubovitz, T. Petrocelli, R. Kotchetkov, M.K. Connor, K. Han, J.H. Lee, S. Ciarallo, C. Catzavelos, R. Beniston, E. Franssen, J.M. Slingerland, PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest, Nat. Med. 8 (2002) 1153–1160.
[127] J. Leger, M. Kempf, G. Lee, R. Brandt, Conversion of serine to aspartate imitates phosphorylation-induced changes in the structure and function of microtubule-associated protein tau, J. Biol. Chem. 272 (1997) 8441–8446.
[128] J. Eidenmuller, T. Fath, T. Maas, M. Pool, E. Sontag, R. Brandt, Phosphorylation-mimicking glutamate clusters in the proline-rich region are sufficient to simulate the functional deficiencies of hyperphosphorylated tau protein, Biochem. J. 357 (2001) 759–767.
[129] T. Fath, J. Eidenmuller, R. Brandt, Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease, J. Neurosci. 22 (2002) 9733–9741.
[130] G.V. Johnson, C.D. Bailey, Tau, where are we now? J. Alzheimer's Dis. 4 (2002) 375–398.
[131] K. Baumann, E.M. Mandelkow, J. Biernat, H. Piwnica-Worms, E. Mandelkow, Abnormal Alzheimer-like phosphorylation of tau-protein by cyclin-dependent kinases cdk2 and cdk5, FEBS Lett. 336 (1993) 417–424.
[132] S. Kobayashi, K. Ishiguro, A. Omori, M. Takamatsu, M. Arioka, K. Imahori, T. Uchida, A cdc2-related kinase PSSALRE/cdk5 is homologous with the 30 kDa subunit of tau protein kinase II, a proline-directed protein kinase associated with microtubule, FEBS Lett. 335 (1993) 171–175.
[133] M.K. Ahlijanian, N.X. Barrezueta, R.D. Williams, A. Jakowski, K.P. Kowsz, S. McCarthy, T. Coskran, A. Carlo, P.A. Seymour, J.E. Burkhardt, R.B. Nelson, J.D. McNeish, Hyperphosphorylated tau and neurofilament and cytoskeletal disruptions in mice overexpressing human p25, an activator of cdk5, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 2910–2915.
[134] F. Bian, R. Nath, G. Sobocinski, R.N. Booher, W.J. Lipinski, M.J. Callahan, A. Pack, K.K. Wang, L.C. Walker, Axonopathy, tau abnormalities, and dyskinesia, but no neurofibrillary tangles in p25-transgenic mice, J. Comp. Neurol. 446 (2002) 257–266.
[135] H. Patzke, U. Maddineni, R. Ayala, M. Morabito, J. Volker, P. Dikkes, M.K. Ahlijanian, L.H. Tsai, Partial rescue of the p35-/- brain phenotype by low expression of a neuronal-specific enolase p25 transgene, J. Neurosci. 23 (2003) 2769–2778.
[136] C. Van den Haute, K. Spittaels, J. Van Dorpe, R. Lasrado, K. Vandezande, I. Laenen, H. Geerts, F. Van Leuven, Coexpression of human cdk5 and its activator p35 with human protein tau in neurons in brain of triple transgenic mice, Neurobiol. Dis. 8 (2001) 32–44.
[137] M. Angelo, F. Plattner, E.E. Irvine, K.P. Giese, Improved reversal learning and altered fear conditioning in transgenic mice with regionally restricted p25 expression, Eur. J. Neurosci. 18 (2003) 423–431.
[138] J.L. Hallows, K. Chen, R.A. DePinho, I. Vincent, Decreased cyclin-dependent kinase 5 (cdk5) activity is accompanied by

redistribution of cdk5 and cytoskeletal proteins and increased cytoskeletal protein phosphorylation in p35 null mice, J. Neurosci. 23 (2003) 10633–10644.

[139] J.C. Cruz, H.C. Tseng, J.A. Goldman, H. Shih, L.H. Tsai, Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles, Neuron 40 (2003) 471–483.

[140] W. Noble, V. Olm, K. Takata, E. Casey, O. Mary, J. Meyerson, K. Gaynor, J. LaFrancois, L. Wang, T. Kondo, P. Davies, M. Burns, Veeranna, R. Nixon, D. Dickson, Y. Matsuoka, M. Ahlijanian, L.F. Lau, K. Duff, Cdk5 is a key factor in tau aggregation and tangle formation in vivo, Neuron 38 (2003) 555–565.

[141] K. Spittaels, C. Van den Haute, J. Van Dorpe, D. Terwel, K. Vandezande, R. Lasrado, K. Bruynseels, M. Irizarry, M. Verhoye, J. Van Lint, J.R. Vandenheede, D. Ashton, M. Mercken, R. Loos, B. Hyman, A. Van der Linden, H. Geerts, F. Van Leuven, Neonatal neuronal overexpression of glycogen synthase kinase-3 beta reduces brain size in transgenic mice, Neuroscience 113 (2002) 797–808.

[142] K. Tilleman, I. Stevens, K. Spittaels, C.V. Haute, S. Clerens, G. Van Den Bergh, H. Geerts, F. Van Leuven, F. Vandesande, L. Moens, Differential expression of brain proteins in glycogen synthase kinase-3 transgenic mice: a proteomics point of view, Proteomics 2 (2002) 94–104.

[143] J.J. Lucas, F. Hernandez, P. Gomez-Ramos, M.A. Moran, R. Hen, J. Avila, Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice, EMBO J. 20 (2001) 27–39.

[144] F. Hernandez, J. Borrell, C. Guaza, J. Avila, J.J. Lucas, Spatial learning deficit in transgenic mice that conditionally over-express GSK-3beta in the brain but do not form tau filaments, J. Neurochem. 83 (2002) 1529–1533.

[145] T. Ishizawa, N. Sahara, K. Ishiguro, J. Kersh, E. McGowan, J. Lewis, M. Hutton, D.W. Dickson, S.H. Yen, Co-localization of glycogen synthase kinase-3 with neurofibrillary tangles and granulovacuolar degeneration in transgenic mice, Am. J. Pathol. 163 (2003) 1057–1067.

[146] R. Nuydens, G. Van Den Kieboom, C. Nolten, C. Verhulst, P. Van Osta, K. Spittaels, C. Van den Haute, E. De Feyter, H. Geerts, F. Van Leuven, Coexpression of GSK-3beta corrects phenotypic aberrations of dorsal root ganglion cells, cultured from adult transgenic mice overexpressing human protein tau, Neurobiol. Dis. 9 (2002) 38–48.

[147] K. Spittaels, C. Van den Haute, J. Van Dorpe, H. Geerts, M. Mercken, K. Bruynseels, R. Lasrado, K. Vandezande, I. Laenen, T. Boon, J. Van Lint, J. Vandenheede, D. Moechars, R. Loos, F. Van Leuven, Glycogen synthase kinase-3beta phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice, J. Biol. Chem. 275 (2000) 41340–41349.

[148] M. Perez, F. Hernandez, F. Lim, J. Diaz-Nido, J. Avila, Chronic lithium treatment decreases mutant tau protein aggregation in a transgenic mouse model, J. Alzheimer's Dis. 5 (2003) 301–308.

[149] R.S. Jope, Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes, Trends Pharmacol. Sci. 24 (2003) 441–443.

[150] G.A. Higgins, H. Jacobsen, Transgenic mouse models of Alzheimer's disease: phenotype and application, Behav. Pharmacol. 14 (2003) 419–438.

[151] M. Kobayashi, K. Ishiguro, Y. Katoh-Fukui, M. Yokoyama, S.C. Fujita, Phosphorylation state of tau in the hippocampus of apolipoprotein E4 and E3 knock-in mice, NeuroReport 14 (2003) 699–702.

[152] F.M. Harris, W.J. Brecht, Q. Xu, I. Tesseur, L. Kekonius, T. Wyss-Coray, J.D. Fish, E. Masliah, P.C. Hopkins, K. Scearce-Levie, K.H. Weisgraber, L. Mucke, R.W. Mahley, Y. Huang, Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10966–10971.

[153] G.M. Gibb, J. Pearce, J.C. Betts, S. Lovestone, M.M. Hoffmann, W. Maerz, W.P. Blackstock, B.H. Anderton, Differential effects of apolipoprotein E isoforms on phosphorylation at specific sites on tau by glycogen synthase kinase-3 beta identified by nano-electrospray mass spectrometry, FEBS Lett. 485 (2000) 99–103.

[154] Y. Huang, X.Q. Liu, T. Wyss-Coray, W.J. Brecht, D.A. Sanan, R.W. Mahley, Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 8838–8843.

[155] N. Ohkubo, Y.D. Lee, A. Morishima, T. Terashima, S. Kikkawa, M. Tohyama, M. Sakanaka, J. Tanaka, N. Maeda, M.P. Vitek, N. Mitsuda, Apolipoprotein E and Reelin ligands modulate tau phosphorylation through an apolipoprotein E receptor/disabled-1/glycogen synthase kinase-3beta cascade, FASEB J. 17 (2003) 295–297.

[156] G. Xu, V. Gonzales, D.R. Borchelt, Abeta deposition does not cause the aggregation of endogenous tau in transgenic mice, Alzheimer Dis. Assoc. Disord. 16 (2002) 196–201.

[157] Y. Tomidokoro, Y. Harigaya, E. Matsubara, M. Ikeda, T. Kawarabayashi, T. Shirao, K. Ishiguro, K. Okamoto, S.G. Younkin, M. Shoji, Brain Abeta amyloidosis in APPsw mice induces accumulation of presenilin-1 and tau, J. Pathol. 194 (2001) 500–506.

[158] M.A. Kurt, D.C. Davies, M. Kidd, K. Duff, D.R. Howlett, Hyperphosphorylated tau and paired helical filament-like structures in the brains of mice carrying mutant amyloid precursor protein and mutant presenilin-1 transgenes, Neurobiol. Dis. 14 (2003) 89–97.

[159] V. Blanchard, S. Moussaoui, C. Czech, N. Touchet, B. Bonici, M. Planche, T. Canton, I. Jedidi, M. Gohin, O. Wirths, T.A. Bayer, D. Langui, C. Duyckaerts, G. Tremp, L. Pradier, Time sequence of maturation of dystrophic neurites associated with Abeta deposits in APP/PS1 transgenic mice, Exp. Neurol. 184 (2003) 247–263.

[160] G. Pigino, G. Morfini, A. Pelsman, M.P. Mattson, S.T. Brady, J. Busciglio, Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport, J. Neurosci. 23 (2003) 4499–4508.

[161] J. Lewis, D.W. Dickson, W.L. Lin, L. Chisholm, A. Corral, G. Jones, S.H. Yen, N. Sahara, L. Skipper, D. Yager, C. Eckman, J. Hardy, M. Hutton, E. McGowan, Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP, Science 293 (2001) 1487–1491.

[162] J. Gotz, F. Chen, J. van Dorpe, R.M. Nitsch, Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils, Science 293 (2001) 1491–1495.

[163] S. Oddo, A. Caccamo, J.D. Shepherd, M.P. Murphy, T.E. Golde, R. Kayed, R. Metherate, M.P. Mattson, Y. Akbari, F.M. LaFerla, Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction, Neuron 39 (2003) 409–421.

[164] A. Boutajangout, M. Authelet, V. Blanchard, N. Touchet, G. Tremp, L. Pradier, J.P. Brion, Characterisation of cytoskeletal abnormalities in mice transgenic for wild-type human tau and familial Alzheimer's disease mutants of APP and presenilin-1, Neurobiol. Dis. 15 (2004) 47–60.

[165] S. Oddo, A. Caccamo, M. Kitazawa, B.P. Tseng, F.M. LaFerla, Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease, Neurobiol. Aging 24 (2003) 1063–1070.

[166] H. Arai, K. Ishiguro, H. Ohno, M. Moriyama, N. Itoh, N. Okamura, T. Matsui, Y. Morikawa, E. Horikawa, H. Kohno, H. Sasaki, K. Imahori, CSF phosphorylated tau protein and mild cognitive impairment: a prospective study, Exp. Neurol. 166 (2000) 201–203.

[167] T.W. Mitchell, E.J. Mufson, J.A. Schneider, E.J. Cochran, J. Nissanov, L.Y. Han, J.L. Bienias, V.M. Lee, J.Q. Trojanowski, D.A. Bennett, S.E. Arnold, Parahippocampal tau pathology in healthy aging, mild cognitive impairment, and early Alzheimer's disease, Ann. Neurol. 51 (2002) 182–189.

[168] N. Ghoshal, F. Garcia-Sierra, J. Wuu, S. Leurgans, D.A. Bennett, R.W. Berry, L.I. Binder, Tau conformational changes correspond to impairments of episodic memory in mild cognitive impairment and Alzheimer's disease, Exp. Neurol. 177 (2002) 475–493.
[169] M. Rapoport, H.N. Dawson, L.I. Binder, M.P. Vitek, A. Ferreira, Tau is essential to beta-amyloid-induced neurotoxicity, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6364–6369.
[170] J. Hardy, The relationship between amyloid and tau, J. Mol. Neurosci. 20 (2003) 203–206.
[171] M.P. Mattson, Oxidative stress, perturbed calcium homeostasis, and immune dysfunction in Alzheimer's disease, J. Neurovirol. 8 (2002) 539–550.