Tau phosphorylation in neuronal cell function and dysfunction

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Summary

Tau is a group of neuronal microtubule-associated proteins that are formed by alternative mRNA splicing and accumulate in neurofibrillary tangles in Alzheimer's disease (AD) brain. Tau plays a key role in regulating microtubule dynamics, axonal transport and neurite outgrowth, and all these functions of tau are modulated by site-specific phosphorylation. There is significant evidence that a disruption of normal phosphorylation events results in tau dysfunction in neurodegenerative diseases, such as AD, and is a contributing factor to the pathogenic processes. Indeed, the abnormal tau phosphorylation that occurs in neurodegenerative conditions not only results in a toxic loss of function (e.g. decreased microtubule binding) but probably also a toxic gain of function (e.g. increased tau-tau interactions). Although tau is phosphorylated in vitro by numerous protein kinases, how many of these actually phosphorylate tau in vivo is unclear. Identification of the protein kinases that phosphorylate tau in vivo in both physiological and pathological processes could provide potential therapeutic targets for the treatment of AD and other neurodegenerative diseases in which there is tau pathology.

Key words: Tau, Microtubules, Phosphorylation, Kinases, Alzheimer's disease

Introduction

It has been almost 30 years since tau was discovered as a heat stable protein that facilitates in vitro microtubule assembly (Weingarten et al., 1975). Following this initial discovery, further studies demonstrated that tau is a phosphoprotein and that phosphorylation negatively regulates its ability to stimulate microtubule assembly (Cleveland et al., 1977; Jameson et al., 1980; Lindwall and Cole, 1984). The breakthrough discovery that catapulted tau into the limelight was the finding that it is the major component of the paired helical filaments (PHFs) that make up the neurofibrillary tangles (NFTs) in Alzheimer's disease (AD) brain, and that the tau in PHFs and NFTs is abnormally phosphorylated (Grundke-Iqbal et al., 1986a; Grundke-Iqbal et al., 1986b; Kosik et al., 1986; Wood et al., 1986). Subsequently, it was reported that microtubule assembly in brain extracts from AD cases is impaired and that the hyperphosphorylation of tau may contribute to this deficit (Iqbal et al., 1986). These findings significantly increased interest in tau and tau phosphorylation both in physiological and in pathological settings. Over the past two decades, much has been learned about tau phosphorylation and function; however, there is still much to learn. Here, we briefly present the basics of tau phosphorylation and then discuss recent work on how site-specific phosphorylation modulates the physiological and pathological roles of tau. More comprehensive overviews of tau can be found elsewhere (Avila et al., 2004; Johnson and Bailey, 2002; Stoothoff and Johnson, 2004).

The essentials of tau phosphorylation

Tau is primarily, although not exclusively, a neuronal protein. In adult human brain, there are six major isoforms of tau generated by alternative mRNA splicing. Tau has 0, 1 or 2 N-terminal inserts (resulting from the splicing in or out of exons 2 and 3) and either 3 or 4 microtubule-binding domains (resulting from the splicing in or out of exon 10) (reviewed in Johnson and Jenkins, 1999) (Fig. 1). The splicing of tau is developmentally regulated, as is its phosphorylation state. In fetal brain, only the shortest tau isoform is present (minus exons 2, 3 and 10) (Kosik et al., 1989) and fetal tau is more extensively phosphorylated than adult tau (Watanabe et al., 1993). Tau from fetal brain promotes microtubule assembly less efficiently than tau from adult brain (Yoshida and Ihara, 1993) and elevated levels of phosphorylated tau correlate with the presence of dynamic microtubules during periods of high plasticity in the developing mammalian brain (Brion et al., 1994).

The longest form of adult human brain tau has 80 Ser or Thr residues and 5 Tyr residues; therefore, almost 20% of the molecule has the potential to be phosphorylated (Goedert et al., 1989). In vitro, tau is a substrate for many protein kinases; however, the number of protein kinases that actually phosphorylate tau in vivo is probably much lower. Dynamic, site-specific phosphorylation of tau is essential for its proper functioning (see below). Furthermore, there is increasing evidence that inappropriate phosphorylation of tau, which leads to tau dysfunction, results in decreased cell viability. Indeed, in all neurodegenerative diseases in which tau pathology has been observed, the tau is abnormally phosphorylated (Lee et al., 2001). These diseases include a group of rare autosomal dominant neurodegenerative diseases collectively known as 'frontotemporal dementia with

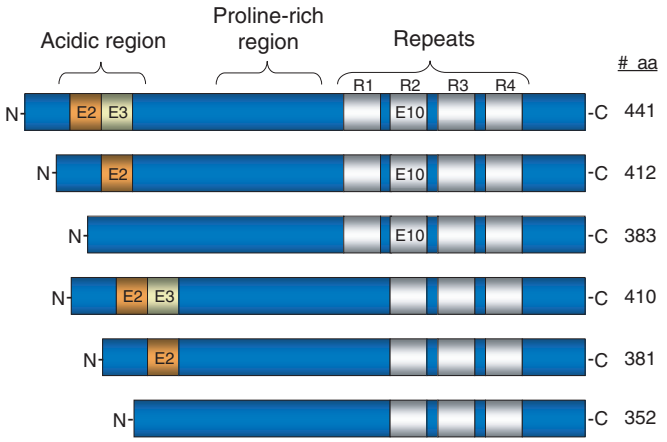

Fig. 1. Schematic diagram showing the organization of the six predominant isoforms of tau found in adult human brain. The number of amino acids (# aa) in each isoform is indicated at the right. The six isoforms are generated by splicing in or out exons 2 and 3 (E2 and E3) in the N-terminal region and exon 10 (E10) in the C-terminal region. The splicing in or out exon 10 results in a tau form with or without the second microtubule-binding repeat (R2), to yield isoforms with four or three microtubule-binding domains, respectively (Goedert et al., 1989). In fetal brain, only the shortest isoform of tau (352 amino acids) is present (Kosik et al., 1989). The proline-rich region is extensively phosphorylated in tau from Alzheimer's disease brain (reviewed by Johnson and Jenkins, 1999). The function of the N-terminal acidic region has not been clearly defined, although it might be involved in regulating the interaction of tau with the plasma membrane (Brandt et al., 1995).

parkinsonism linked to chromosome 17 (FTDP-17), which are caused by mutations in the tau gene located on chromosome 17q21 (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). These and other findings support the hypothesis that the aberrant phosphorylation of tau plays a role in the pathogenesis of these diseases.

Kinases that regulate tau phosphorylation in vivo

Although many potential 'tau kinases' have been examined, thus far only a few are considered to be good candidates for bona fide in vivo tau kinases. One likely tau kinase is glycogen synthase kinase 3β (GSK3β) (reviewed by Doble and Woodgett, 2003). GSK3β is expressed at high levels in brain (Woodgett, 1990), where it localizes to neurons (Leroy and Brion, 1999), and thus is in an appropriate compartment to access tau. GSK3β associates with microtubules (Ishiguro et al., 1993) and, when this kinase is overexpressed in cells, the phosphorylation state of tau dramatically increases at numerous sites (Cho and Johnson, 2003; Lovestone et al., 1996; Wagner et al., 1996). Immunoblot analyses have revealed that modest (20-50%) increases in expression of GSK3β in the brains of transgenic mice result in increased tau phosphorylation at several sites (Lucas et al., 2001; Spittaels et al., 2000). Furthermore, treatment of cells with lithium, a selective inhibitor of GSK3 (reviewed by Jope, 2003), significantly attenuates tau phosphorylation (Hong et al., 1997; Lovestone et al., 1999; Stambolic et al., 1996). Chronic lithium treatment also decreases tau phosphorylation in a mouse model in which mutant FTDP-17 tau is overexpressed (Perez et al., 2003). These and other findings provide extremely strong evidence that tau is an in vivo substrate of GSK3β, and that abnormal phosphorylation of tau by GSK3β might contribute to the pathogenic processes in AD brain. However, GSK3β phosphorylates and regulates numerous other proteins (Jope and Johnson, 2004), and therefore the possibility remains that GSK3β indirectly regulates tau phosphorylation in vivo. Nonetheless, of all the protein kinases known to phosphorylate tau in vitro, the strongest evidence so far is for tau being a substrate of GSK3β in vivo.

Another possible in vivo tau kinase is cyclin-dependent kinase 5 (Cdk5). Cdk5 is a unique member of the Cdk family that is activated by interaction with the non-cyclins, p35 and p39, which are regulatory proteins that are expressed almost exclusively in postmitotic neurons (Dhavan and Tsai, 2001; Shelton and Johnson, 2004). p35 and p39 can be proteolyzed by the calcium-dependent protease calpain (Kusakawa et al., 2000; Patzke and Tsai, 2002), resulting in p25 and p29, respectively, which are more stable than p35 or p39 and thus cause a more prolonged activation of Cdk5 (Kusakawa et al., 2000; Patrick et al., 1998; Patrick et al., 1999; Patzke and Tsai, 2002). In vitro, tau is a substrate of Cdk5, and most if not all of the sites on tau that are phosphorylated by Cdk5 are also phosphorylated by GSK3β (Anderton et al., 2001; Hashiguchi et al., 2002; Liu et al., 2002; Lund et al., 2001). Overexpression of Cdk5 and p25, but not p35, results in increased tau phosphorylation at specific sites (Hamdane et al., 2003; Patrick et al., 1999). However, other studies have found that upregulation of Cdk5 and p25 increases Cdk5 activity but does not significantly increase tau phosphorylation in situ (Kerokoski et al., 2002; Shelton et al., 2004). In addition, inhibition of Cdk5 in primary cortical neurons increases tau phosphorylation (Morfini et al., 2004).

Expression of p25 in one transgenic mouse model resulted in a significant increase in Cdk5 activity, and immunohistochemistry revealed increased staining by phospho-dependent tau antibodies, although no increase in tau phosphorylation was evident on immunoblots (Ahlijanian et al., 2000). However, in another transgenic mouse model in which p25 was overexpressed, increased phosphorylation of tau was apparent both on immunoblots and by immunohistochemical staining – as demonstrated by reactivity with the phospho-dependent anti-tau antibodies AT8 (which recognizes phospho-Ser202/Thr205) and PHF-1 (which recognizes phospho-Ser396/Ser404) (Cruz et al., 2003). Crossing a p25-overexpressing mouse (Ahlijanian et al., 2000) with a mouse expressing FTDP-17 P301L-mutant tau also enhanced tau phosphorylation (Noble et al., 2003). Interestingly, in this double-transgenic mouse, there is increased phospho-Tyr216 GSK3β immunoreactivity, and phospho-Tyr216 GSK3β and phospho-tau epitopes co-localize. Although not necessary for GSK3β activity, phosphorylation at Tyr216, which is probably a result of autophosphorylation, does increase the activity of GSK3β, perhaps in part by increasing the stability of the kinase (Cole et al., 2004; Hughes et al., 1993). These findings suggest that increased Cdk5 activity results in an increase in GSK3β tyrosine phosphorylation. However, the levels of phospho-Tyr216 GSK3β and total GSK3β levels were not quantitated in this study (Noble et al., 2003), and thus the apparent increase in phospho-Tyr216 GSK3β immunoreactivity could be due to an

upregulation in overall GSK3β levels. Nonetheless, these data suggest that GSK3β contributes to the increased tau phosphorylation in these mice.

Although increasing Cdk5 activity by overexpressing p25 results in increased tau phosphorylation, knocking out p35, which significantly decreases Cdk5 activity, also increases tau phosphorylation (Hallows et al., 2003). Intriguingly, Hallows et al. demonstrated that GSK3β activity is increased by >50% in p35-knockout mice (Hallows et al., 2003), again implicating GSK3β as the kinase that phosphorylates tau. This is similar to the observation of Morfini et al. in neuronal cell models: inhibition of Cdk5 leads to activation of GSK3 (Morfini et al., 2004).

Considering the conflicting nature of these reports, the role of Cdk5 in the regulation of tau phosphorylation needs to be reconsidered. First, these data indicate that, under physiological conditions when p35 (or p39) is the predominant, if not exclusive, regulator (Smith et al., 2001), Cdk5 might not directly phosphorylate tau. Indeed, overexpression of tau, Cdk5 and p35 in mice does not increase tau phosphorylation (Van den Haute et al., 2001), which contrasts with what is observed when p25 is overexpressed (Cruz et al., 2003). This may be because the subcellular localization of p35, the activator that predominates in physiological conditions, is tightly regulated (Smith et al., 2001); hence it might not co-localize with tau and thus Cdk5 would not be able to phosphorylate tau. By contrast, in pathological conditions, p25 does not appear to be localized to specific compartments (Smith et al., 2001), and this might allow Cdk5 to access tau, resulting in aberrant phosphorylation.

Cdk5 might also indirectly regulate the kinases and phosphatases that act on tau. Cdk5 phosphorylates two protein phosphatase 1 (PP1) inhibitors, I-1 and I-2 (Agarwal-Mawal and Paudel, 2001; Bibb et al., 2001; Huang and Paudel, 2000). Cdk5 phosphorylation activates I-1 (Bibb et al., 2001; Huang and Paudel, 2000). Because PP1 can dephosphorylate tau (Gong et al., 1994; Liao et al., 1998), this should enhance tau phosphorylation. However, phosphorylation of I-2 by Cdk5 prevents it from inhibiting PP1 (Agarwal-Mawal and Paudel, 2001) and this might result in a shift towards tau dephosphorylation. The activity of GSK3β is inhibited by Ser9 phosphorylation (Sutherland et al., 1993) and a recent study has demonstrated that inhibition of Cdk5 leads to PP1 activation and subsequent dephosphorylation and activation of GSK3β, the net result being increased phosphorylation of tau and kinesin light chain (Morfini et al., 2004). A similar scenario might occur in the p35-knockout mice, where increased GSK3β activity and tau phosphorylation is observed (Hallows et al., 2003). Although there is good evidence that Cdk5 regulates tau phosphorylation in vivo, it remains to be determined whether this is predominantly a direct or indirect effect.

Although the majority of sites on tau that are phosphorylated are Ser/Thr-Pro sites, Ser and Thr sites not followed by Pro residues are also phosphorylated. Data indicate that tau is phosphorylated by cAMP-dependent protein kinase (PKA) in vivo. For example, treatment of brain slices (Fleming and Johnson, 1995) or cultured cells (Litersky et al., 1996) with forskolin (an adenylyl cyclase activator) and rolipram (a cAMP phosphodiesterase inhibitor) results in a pronounced increase in tau phosphorylation. Activation of endogenous PKA can thus increase tau phosphorylation. Furthermore, many of the sites on tau that are phosphorylated in brain slices in response to increases in cAMP levels are those that are phosphorylated by PKA in vitro (Fleming and Johnson, 1995).

Microtubule-affinity-regulating kinase (MARK) might also regulate tau phosphorylation in vivo. MARK selectively phosphorylates a KXGS motif, which is present in each microtubule-binding repeat of tau, as well as other microtubule-associated proteins (Drewes et al., 1997; Drewes et al., 1995). Because tau is phosphorylated at KXGS motifs in vivo (Ser262 being the most prominently phosphorylated KXGS motif) (Seubert et al., 1995), and MARK probably phosphorylates these epitopes more efficiently in situ than do other protein kinases (Biernat et al., 2002), MARK could be a tau kinase. In cultured cell models, MARK appears to regulate tau phosphorylation (Biernat et al., 2002) and, in AD brain, MARK is associated with NFTs and co-localizes with phospho-Ser262 staining (Chin et al., 2000). Although the ability of MARK to phosphorylate tau in a mouse model has not been examined directly, MARK phosphorylates tau at KXGS motifs in retinal ganglion cell axons (Mandelkow et al., 2004). Furthermore, Nishimara et al. also recently showed that PAR-1 (the *Drosophila* homologue of MARK) probably phosphorylates tau in vivo in flies (Nishimura et al., 2004).

Overall, it is clear that GSK3β, Cdk5, PKA and MARK probably modulate tau phosphorylation in vivo at some level, either directly or indirectly. Many other kinases can increase tau phosphorylation in non-neuronal cell model systems when they are overexpressed. However, without validation in a true neuronal system in which the proteins are expressed in the appropriate context, the role of these kinases in modulating tau phosphorylation in vivo remain to be established.

The physiological role of tau phosphorylation

Microtubule binding

The phosphorylation of tau at specific sites is the predominant mechanism by which tau function is regulated. The ability to bind and stabilize microtubules is a hallmark of tau, and it is becoming increasingly apparent that phosphorylation of a few specific sites plays a significant role in regulating tau-microtubule interactions. Phosphorylation of the KXGS motifs within the microtubule-binding repeats of tau strongly reduces the binding of tau to microtubules in vitro (Biernat et al., 1993) and probably in vivo (Biernat and Mandelkow, 1999; Drewes et al., 1995). In vitro studies have shown that phosphorylation of Ser262 alone is sufficient to attenuate significantly the ability of tau to bind microtubules in vitro (Biernat et al., 1993); however, in situ phosphorylation of two or more KXGS motifs (especially Ser262 and Ser356) is required to decrease microtubule binding and facilitate the formation of cell processes (Biernat and Mandelkow, 1999). MARK, PKA and calcium/calmodulin-dependent protein kinase II might contribute to the phosphorylation of these sites in vivo (Drewes et al., 1995; Litersky et al., 1996; Scott et al., 1993; Sironi et al., 1998).

Phosphorylation of Thr231 also plays a significant role in regulating tau-microtubule interactions. Thr231 is a primed GSK3β site on tau, which means that Ser235 must be phosphorylated first to get efficient phosphorylation of Thr231 (Goedert et al., 1994). Phosphorylation of Thr231 greatly

diminishes the ability of tau to bind microtubules in situ (Cho and Johnson, 2003). Furthermore, when cell lysates are separated into soluble cytosolic and insoluble cytoskeletal fractions, almost all the tau that is phosphorylated at the Thr231 epitope is present in the soluble fraction (Cho and Johnson, 2003; Hamdane et al., 2003). In addition, phosphorylation of Thr231 inhibits the ability of tau to stabilize microtubules in the cell as indicated by a reduction in the levels of acetylated tubulin, a marker of microtubule stability (Cho and Johnson, 2004). By contrast, phosphorylation of tau at Ser396 and/or Ser404 does not significantly affect the ability of tau to bind to microtubules (Cho and Johnson, 2003). Phosphorylation of Thr231 thus appears to play a key role in regulating tau function in vivo.

Neurite outgrowth

Tau probably plays a key role in the regulation of neurite extension. Early studies demonstrated that suppression of tau expression in cultured cerebellar neurons by using antisense oligonucleotides significantly suppresses neurite outgrowth (Caceres and Kosik, 1990; Caceres et al., 1991). However, a tau-knockout mouse has no overt phenotype except for a decrease in the number of microtubules in small-caliber axons (Harada et al., 1994). This lack of phenotype is probably due to a redundancy of function and/or compensatory upregulation of other microtubule-associated proteins (Dawson et al., 2001; Harada et al., 1994). Indeed, knocking out both MAP-1B and tau results in a severe dysgenesis of axonal tracts (corpus callosum, anterior commissure, etc.), delayed neuronal migration resulting in a disruption of neuronal layer formation and disorganization of microtubules in growth cones (Takei et al., 2000). Furthermore, primary cultures of hippocampal neurons lacking tau exhibit decreased rates of neurite extension and inhibited neuronal polarization (i.e. the development of axons and dendrites) (Dawson et al., 2001), defects that are more pronounced in the tau-MAP-1B double-knockout model (Takei et al., 2000).

During axonogenesis, tau function appears to be locally regulated by phosphorylation. Interestingly, there is a proximo-distal gradient in tau phosphorylation at Ser199/202 and Thr205 along the nascent axon: tau in the cell body and proximal axon is ~80% phosphorylated at these sites; and tau in the growth cone is ~20% phosphorylated (Mandell and Banker, 1996). Furthermore, neurite outgrowth seems to require tau phosphorylation at KXGS motifs in a specific spatial and temporal manner, probably by MARK or PKA (Biernat and Mandelkow, 1999; Biernat et al., 2002). Conversely, there is indirect evidence that GSK3-mediated tau phosphorylation might facilitate neurite retraction (Sayas et al., 2002).

Axonal transport

Tau also regulates axonal transport. In mouse models in which tau is overexpressed in the central nervous system, there is almost always axonopathy, predominantly in spinal cord neurons (Ishihara et al., 1999; Probst et al., 2000; Spittaels et al., 1999). In these tau-overexpression mouse models, there is invariably evidence of axonal and myelin degeneration with axonal swellings that contain cytoskeletal elements (Ishihara et al., 1999; Probst et al., 2000; Spittaels et al., 1999). In addition, overexpression of the shortest human tau isoform significantly inhibits fast axonal transport in ventral root axons (Ishihara et al., 1999).

Tau can inhibit kinesin-dependent fast axonal transport in cell culture models (Ebneth et al., 1998; Mandelkow et al., 2003; Stamer et al., 2002), and this is probably the case in vivo when tau is overexpressed. The primary mechanism by which tau inhibits kinesin-dependent transport is by reducing the attachment frequency of the motors. Tau has no effect on the speed or run length of kinesin once it is attached to the microtubules (Seitz et al., 2002). Phosphorylation of tau modulates its affinity for microtubules and thus its ability to regulate motor activity. Overexpression of GSK3β in mice transgenic for human tau significantly increases the phosphorylation state of tau and reduces the axonopathy compared with that in mice that overexpress human tau only. In the double-transgenic mice, there is also less motor impairment when compared with the transgenics overexpressing the human tau alone (Spittaels et al., 2000). This is probably because the increase in tau phosphorylation due to overexpression of GSK3β decreases the affinity of tau for microtubules. This should make the tau in the GSK3β-human tau double-transgenic mice less effective at competing with kinesin for binding sites, the net result being greater kinesin binding and a restoration of anterograde axonal transport. Tatebayashi et al. recently demonstrated that, in cell culture models, GSK3β-mediated tau phosphorylation is associated with proper anterograde organelle transport (Tatebayashi et al., 2004), providing further evidence that the control of axonal transport by tau is regulated by GSK3β-mediated phosphorylation.

The pathological effects of tau phosphorylation

Aberrant phosphorylation is a key feature of tau isolated from the brains of individuals with AD and many other diseases exhibiting tau pathology. Since site-specific phosphorylation clearly modulates the function and intracellular localization of tau, inappropriate phosphorylation is probably a key event in the development of tau pathology. Below, we highlight some of the findings relative to the pathological effects of tau phosphorylation. More-comprehensive overviews of the role of abnormal tau phosphorylation in disease pathogenesis can be found elsewhere (Avila et al., 2004; Iqbal et al., 2002; Shahani and Brandt, 2002).

In vitro, pseudophosphorylation (changing Ser to Glu) of Ser396 and Ser404 generates tau that is more fibrillogenic (Abraha et al., 2000), and a tau construct in which Ser422 is mutated to Glu shows a significantly increased propensity to aggregate (Haase et al., 2004). This is intriguing because Aβ treatment of cultured cells causes tau to aggregate, but mutation of Ser422 to Ala prevents the Aβ-induced aggregation (Ferrari et al., 2003). Aβ is a peptide that is produced by the proteolytic processing of amyloid precursor protein (APP) and is the major component of the senile plaques in AD brain. Hence, these findings demonstrate a connection between the two major pathologies in AD brain. Indeed, there is significant evidence that Aβ toxicity is upstream of tau pathology in AD (Hardy, 2003).

Given these findings, phosphorylation of Ser422 may thus play a key role in tau filament formation in vivo. Antibodies to phospho-Ser422 strongly label tau from AD brain, but only

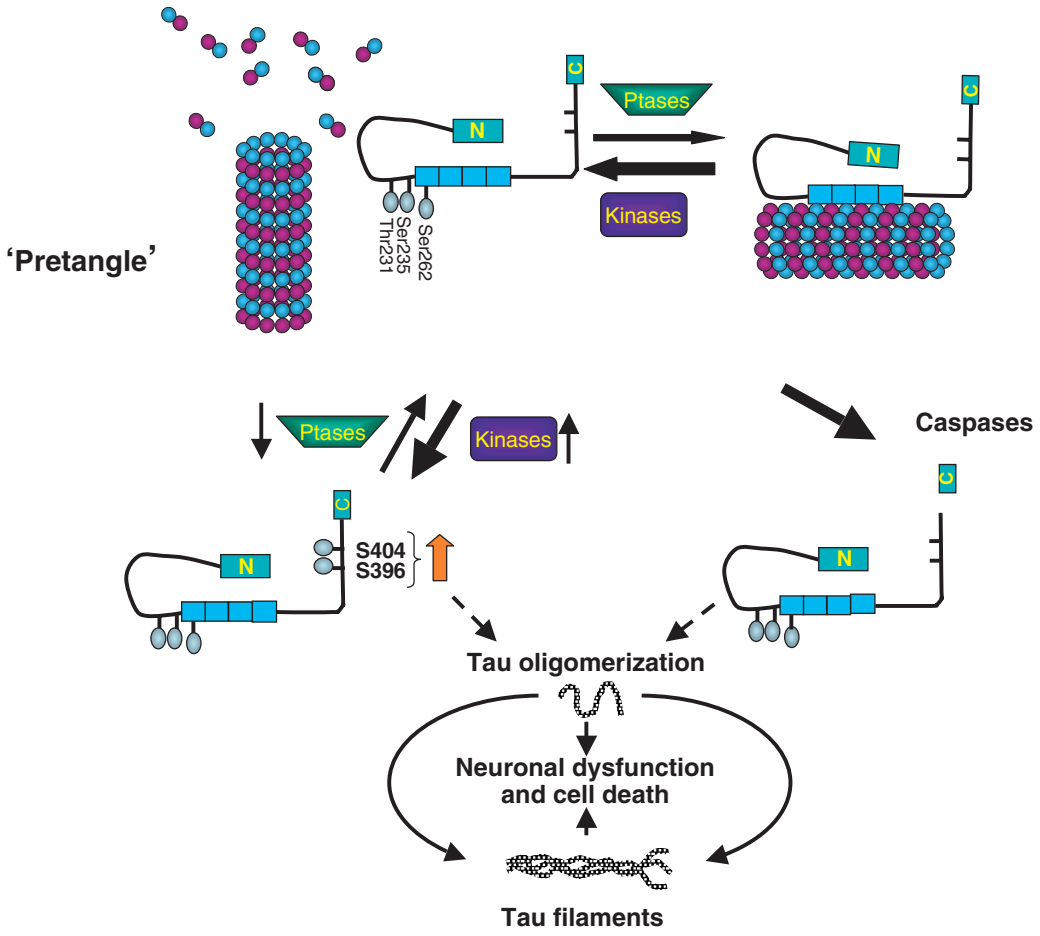

Fig. 2. In the development of tau pathology, tau phosphorylation events are probably sequential. In the early stages of pathology ('pretangle'), the predominant phosphorylation events are probably those that decrease the ability of tau to bind microtubules rather than those that increase the ability of tau to self-associate; this might be caused by an imbalance in the activity of specific protein kinases or phosphatases (Ptases). For example, pretangle neurons in Alzheimer's disease brain are labelled with antibodies that recognize phospho-Thr231 and phospho-Ser262 (Augustinack et al., 2002) and phosphorylation of both of these sites significantly decreases interactions of tau with microtubules (Biernat et al., 1993; Cho and Johnson, 2003). Subsequently, tau can be cleaved by caspase and/or phosphorylated at additional sites such as Ser422 (Ferrari et al., 2003; Haase et al., 2004) and Ser396/404 (Abraha et al., 2000), which increases the propensity of tau to oligomerize and eventually form filamentous aggregates. The exact role that tau oligomers and filaments play in the cell dysfunction/death process has not yet been clearly defined.

weakly label tau from fetal or normal adult human brain (Hasegawa et al., 1996). In AD brain, the phospho-Ser422 antibody predominantly labels intraneuronal NFTs in neurons that have lost their integrity, as well as extra-neuronal NFTs (Augustinack et al., 2002). Interestingly, staining of 'pretangle' neurons with the phospho-Ser422 antibody is rare, indicating that the phosphorylation of this site is not an early event in tau pathology. By contrast, pretangle neurons are stained with antibodies that recognize phospho-Ser262 and phospho-Thr231 (Augustinack et al., 2002), indicating that increased phosphorylation of these sites might be an early event in tau pathology. On the basis of these findings, one can speculate that the initiating phosphorylation events in the evolution of tau pathology are those that decrease the affinity of tau for microtubules, increasing the 'free' pool of tau, and are followed by phosphorylation of sites that make tau more fibrillogenic. However, it should be noted that, although phosphorylation of Ser262 (and Ser214) on tau decreases the affinity of tau for microtubules, these phosphorylation events inhibit tau polymerization into filaments (Schneider et al., 1999). Therefore, not all tau phosphorylation events that lead to decreased microtubule binding contribute to the development of tau pathology. Note also that the cleavage of tau by caspases (Gamblin et al., 2003; Rohn et al., 2002) might synergize with abnormal phosphorylation events to drive tau polymerization in AD brain (Fig. 2). The fact that tau that has been cleaved by caspase is more fibrillogenic than full-length tau supports this hypothesis (Gamblin et al., 2003).

The protein kinases that contribute to the pathological phosphorylation of tau in AD and other neurodegenerative diseases remain elusive. There is some evidence that GSK3β contributes to tau pathology in AD brain (Jope and Johnson, 2004). It has also been reported that GSK3β facilitates APP proteolysis to generate Aβ (Su et al., 2004). However, this finding is somewhat controversial because another group demonstrated that the GSK3α isoform is responsible for facilitating the production of Aβ (Phiel et al., 2003). Nonetheless, it is intriguing to note that treatment of neuronal cells with Aβ results in increased GSK3 activity and enhanced tau phosphorylation (Busciglio et al., 1995; Takashima et al.,

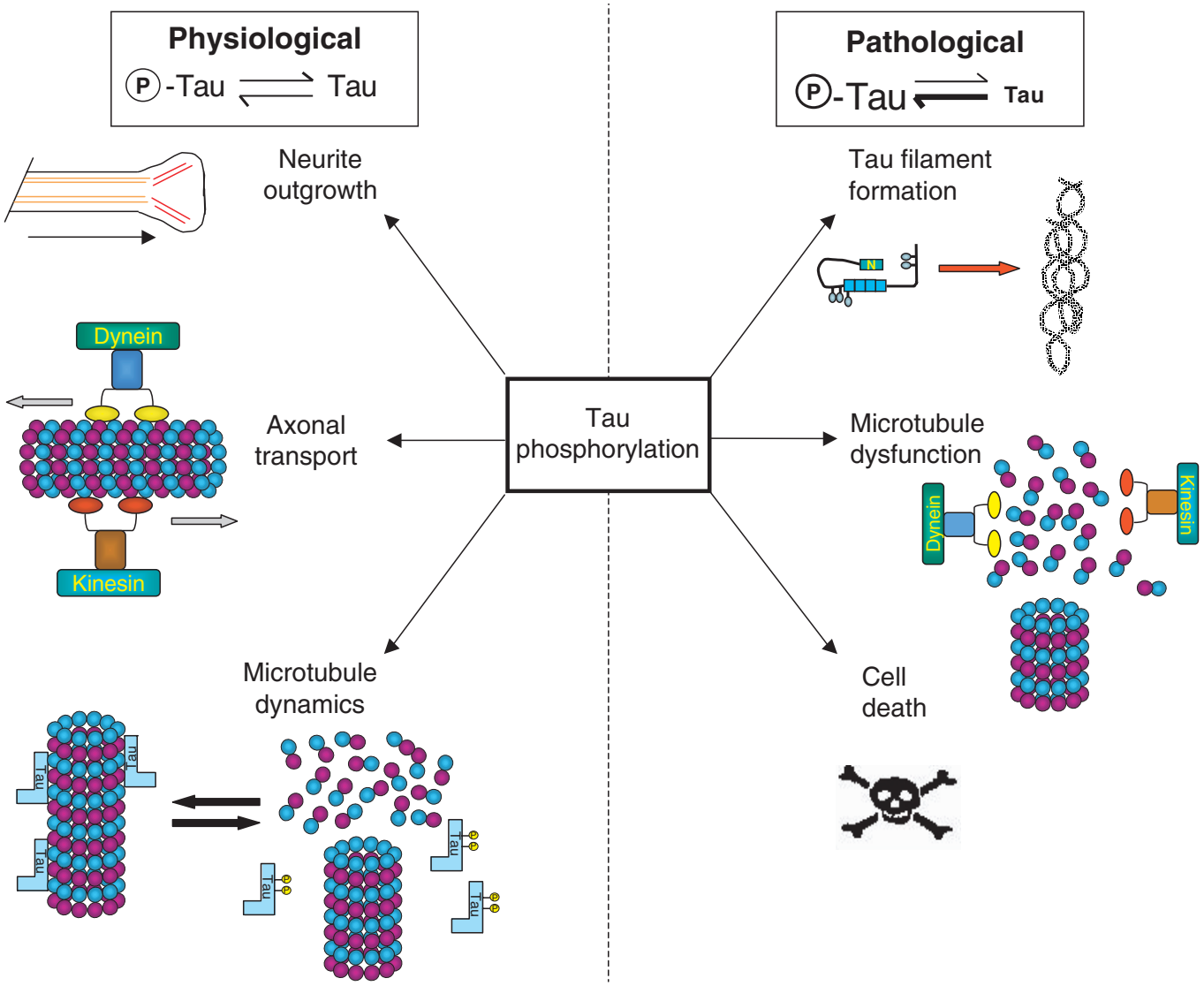

Fig. 3. Tau phosphorylation plays both physiological and pathological roles in the cell. When the phosphorylation state of tau is appropriately coordinated, it plays a role in regulating neurite outgrowth (Biernat and Mandelkow, 1999; Biernat et al., 2002), axonal transport (Spittaels et al., 2000; Tatebayashi et al., 2004) and microtubule stability and dynamics (Cho and Johnson, 2004). However, in pathological conditions in which there is an imbalance in the phosphorylation/dephosphorylation of tau, aberrant tau phosphorylation can cause tau filament formation (Abraha et al., 2000), disrupt microtubule-based processes owing to decreased microtubule binding (Lu and Wood, 1993) and perhaps even increase cell death (Fath et al., 2002).

1996; Takashima et al., 1993). Therefore, we can speculate that, in AD, increases in Aβ production result in increases in GSK3 activity, which facilitates Aβ production and subsequently sustained increases in GSK3 activity, more tau phosphorylation and eventually a pathogenic spiral that results in neuronal degeneration.

An important question that remains unanswered is what role aberrant tau phosphorylation plays in the pathological cascade of events that results in neuronal dysfunction and death both in AD and in the tauopathies. Does a phosphorylation-induced decrease in tau-microtubule binding contribute to cell dysfunction? Is it the formation of insoluble tau aggregates or are there other processes disrupted by abnormal tau phosphorylation that are pathogenic? Numerous hypotheses have been put forth; however, the exact role that tau hyperphosphorylation plays in pathogenic processes remains unclear. Perhaps hyperphosphorylated tau exerts its toxic effects simply by sequestering normal tau into the NFTs, which results in decreases in microtubule stability and axonal transport (Alonso et al., 1996). Consider also that tau pathology might not be central to the disease process. For example, in a triple-transgenic mouse model that expresses mutant presenilin 1, mutant APP and FTDP-17 P301L-mutant tau, both Aβ-containing plaques and NFTs (containing phosphorylated tau) are present; however, it is the accumulation of intracellular Aβ that correlates with defects in synaptic plasticity, not the accumulation of phosphorylated tau (Oddo et al., 2003). Nonetheless, data suggest that tau plays a key role in the pathogenic cascades. For example, neurons from tau-knockout mice are resistant to Aβ-induced

neurotoxicity (Rapoport et al., 2002), and expression of pseudophosphorylated tau constructs in cells is toxic (Fath et al., 2002). Clearly, further investigations are required to clarify the role of tau phosphorylation in the pathogenesis of neurodegenerative diseases.

Conclusions and future directions

Over the past several years, significant progress has been made in our understanding of how phosphorylation regulates tau function. It is evident that specific, coordinated phosphorylation events are crucial for appropriate neurite outgrowth and axonal transport processes (Fig. 3). However, it still remains to be determined which protein kinases phosphorylate tau in vivo and how the dynamics of these processes are regulated. It is also evident that tau is abnormally phosphorylated when tau pathology occurs in a neurodegenerative disease. Given that the phosphorylation of specific sites on tau can inhibit its ability to bind microtubules efficiently and increase its ability to polymerize, it is likely the abnormal phosphorylation events play a role in tau pathogenic processes (Figs 2 and 3). It is also likely that the phosphorylation-induced loss of function (i.e. impairment of microtubule binding), as well as the toxic gain of function (i.e. an increased propensity to oligomerize), synergize to reduce the levels of functional tau and thus disrupt normal microtubule-based functions, which could contribute to the demise of the cell (Figs 2 and 3). Nevertheless, further studies are needed to clarify the role of abnormal tau phosphorylation in the pathogenic cascades in AD, as well as the protein kinases that directly phosphorylate tau in both the normal and disease states.

References

Abraha, A., Ghoshal, N., Gamblin, T. C., Cryns, V., Berry, R. W., Kuret, J. and Binder, L. I. (2000). C-terminal inhibition of tau assembly in vitro and in Alzheimer's disease. *J. Cell Sci.* **113**, 3737-3745.

Agarwal-Mawal, A. and Paudel, H. K. (2001). Neuronal Cdc2-like protein kinase (Cdk5/p25) is associated with protein phosphatase 1 and phosphorylates inhibitor-2. *J. Biol. Chem.* **276**, 23712-23718.

Ahlijanian, M. K., Barrezueta, N. X., Williams, R. D., Jakowski, A., Kowsz, K. P., McCarthy, S., Coskran, T., Carlo, A., Seymour, P. A., Burkhardt, J. E. et al. (2000). Hyperphosphorylated tau and neurofilament and cytoskeletal disruptions in mice overexpressing human p25, an activator of cdk5. *Proc. Natl. Acad. Sci. USA* **97**, 2910-2915.

Alonso, A. C., Grundke-Iqbal, I. and Iqbal, K. (1996). Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat. Med.* **2**, 783-787.

Anderton, B. H., Betts, J., Blackstock, W. P., Brion, J. P., Chapman, S., Connell, J., Dayanandan, R., Gallo, J. M., Gibb, G., Hanger, D. P. et al. (2001). Sites of phosphorylation in tau and factors affecting their regulation. *Biochem. Soc. Symp.* **67**, 73-80.

Augustinack, J. C., Schneider, A., Mandelkow, E. M. and Hyman, B. T. (2002). Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* **103**, 26-35.

Avila, J., Lucas, J. J., Perez, M. and Hernandez, F. (2004). Role of tau protein in both physiological and pathological conditions. *Physiol. Rev.* **84**, 361-384.

Bibb, J. A., Nishi, A., O'Callaghan, J. P., Ule, J., Lan, M., Snyder, G. L., Horiuchi, A., Saito, T., Hisanaga, S., Czernik, A. J. et al. (2001). Phosphorylation of protein phosphatase inhibitor-1 by Cdk5. *J. Biol. Chem.* **276**, 14490-14497.

Biernat, J. and Mandelkow, E. M. (1999). 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**, 727-740.

Biernat, J., Gustke, N., Drewes, G., Mandelkow, E. M. and Mandelkow, E. (1993). Phosphorylation of Ser262 strongly reduces binding of tau to microtubules: distinction between PHF-like immunoreactivity and microtubule binding. *Neuron* **11**, 153-163.

Biernat, J., Wu, Y. Z., Timm, T., Zheng-Fischhofer, Q., Mandelkow, E., Meijer, L. and Mandelkow, E. M. (2002). Protein kinase MARK/PAR-1 is required for neurite outgrowth and establishment of neuronal polarity. *Mol. Biol. Cell* **13**, 4013-4028.

Brandt, R., Leger, J. and Lee, G. (1995). Interaction of tau with the neural plasma membrane mediated by tau's amino terminal projection domain. *J. Cell Biol.* **131**, 1327-1340.

Brion, J. P., Octave, J. N. and Couck, A. M. (1994). Distribution of the phosphorylated microtubule-associated protein tau in developing cortical neurons. *Neuroscience* **63**, 895-909.

Busciglio, J., Lorenzo, A., Yeh, J. and Yankner, B. A. (1995). beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron* **14**, 879-888.

Caceres, A. and Kosik, K. S. (1990). Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature* **343**, 461-463.

Caceres, A., Potrebic, S. and Kosik, K. S. (1991). The effect of tau antisense oligonucleotides on neurite formation of cultured cerebellar macroneurons. *J. Neurosci.* **11**, 1515-1523.

Chin, J. Y., Knowles, R. B., Schneider, A., Drewes, G., Mandelkow, E. M. and Hyman, B. T. (2000). Microtubule-affinity regulating kinase (MARK) is tightly associated with neurofibrillary tangles in Alzheimer brain: a fluorescence resonance energy transfer study. *J. Neuropathol. Exp. Neurol.* **59**, 966-971.

Cho, J. H. and Johnson, G. V. (2003). Glycogen synthase kinase 3beta phosphorylates tau at both primed and unprimed sites. Differential impact on microtubule binding. *J. Biol. Chem.* **278**, 187-193.

Cho, J. H. and Johnson, G. V. (2004). 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**, 349-358.

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

Cole, A., Frame, S. and Cohen, P. (2004). Further evidence that the tyrosine phosphorylation of glycogen synthase kinase-3 (GSK3) in mammalian cells is an autophosphorylation event. *Biochem. J.* **377**, 249-255.

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

Dawson, H. N., Ferreira, A., Eyster, M. V., Ghoshal, N., Binder, L. I. and Vitek, M. P. (2001). Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J. Cell Sci.* **114**, 1179-1187.

Dhavan, R. and Tsai, L. H. (2001). A decade of CDK5. *Nat. Rev. Mol. Cell Biol.* **2**, 749-759.

Doble, B. W. and Woodgett, J. R. (2003). GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell Sci.* **116**, 1175-1186.

Drewes, G., Trinczek, B., Illenberger, S., Biernat, J., Schmitt-Ulms, G., Meyer, H. E., Mandelkow, E. M. and Mandelkow, E. (1995). Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262. *J. Biol. Chem.* **270**, 7679-7688.

Drewes, G., Ebneth, A., Preuss, U., Mandelkow, E. M. and Mandelkow, E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* **89**, 297-308.

Ebneth, A., Godemann, R., Stamer, K., Illenberger, S., Trinczek, B. and Mandelkow, E. (1998). Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J. Cell Biol.* **143**, 777-794.

Fath, T., Eidenmuller, J. and Brandt, R. (2002). Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease. *J. Neurosci.* **22**, 9733-9741.

Ferrari, A., Hoerndli, F., Baechi, T., Nitsch, R. M. and Gotz, J. (2003). beta-Amyloid induces paired helical filament-like tau filaments in tissue culture. *J. Biol. Chem.* **278**, 40162-40168.

Fleming, L. M. and Johnson, G. V. (1995). Modulation of the phosphorylation state of tau in situ: the roles of calcium and cyclic AMP. *Biochem. J.* **309**, 41-47.

Gamblin, T. C., Chen, F., Zambrano, A., Abraha, A., Lagalwar, S., Guillozet, A. L., Lu, M., Fu, Y., Garcia-Sierra, F., LaPointe, N. et al. (2003). Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **100**, 10032-10037.

Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D. and Crowther, R. A. (1989). Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* **3**, 519-526.

Goedert, M., Jakes, R., Crowther, R. A., Cohen, P., Vanmechelen, E., Vandermeeren, M. and Cras, P. (1994). Epitope mapping of monoclonal antibodies to the paired helical filaments of Alzheimer's disease: identification of phosphorylation sites in tau protein. *Biochem. J.* **301**, 871-877.

Gong, C. X., Grundke-Iqbal, I., Damuni, Z. and Iqbal, K. (1994). Dephosphorylation of microtubule-associated protein tau by protein phosphatase-1 and -2C and its implication in Alzheimer disease. *FEBS Lett.* **341**, 94-98.

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

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M. and Binder, L. I. (1986b). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. USA* **83**, 4913-4917.

Haase, C., Stieler, J. T., Arendt, T. and Holzer, M. (2004). Pseudophosphorylation of tau protein alters its ability for self-aggregation. *J. Neurochem.* **88**, 1509-1520.

Hallows, J. L., Chen, K., DePinho, R. A. and Vincent, I. (2003). 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**, 10633-10644.

Hamdane, M., Sambo, A. V., Delobel, P., Begard, S., Violleau, A., Delacourte, A., Bertrand, P., Benavides, J. and Buee, L. (2003). Mitotic-like tau phosphorylation by p25-Cdk5 kinase complex. *J. Biol. Chem.* **278**, 34026-34034.

Harada, A., Oguchi, K., Okabe, S., Kuno, J., Terada, S., Ohshima, T., Sato-Yoshitake, R., Takei, Y., Noda, T. and Hirokawa, N. (1994). Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature* **369**, 488-491.

Hardy, J. (2003). The relationship between amyloid and tau. *J. Mol. Neurosci.* **20**, 203-206.

Hasegawa, M., Jakes, R., Crowther, R. A., Lee, V. M., Ihara, Y. and Goedert, M. (1996). Characterization of mAb AP422, a novel phosphorylation-dependent monoclonal antibody against tau protein. *FEBS Lett.* **384**, 25-30.

Hashiguchi, M., Saito, T., Hisanaga, S. and Hashiguchi, T. (2002). Truncation of CDK5 activator p35 induces intensive phosphorylation of Ser202/Thr205 of human tau. *J. Biol. Chem.* **277**, 44525-44530.

Hong, M., Chen, D. C., Klein, P. S. and Lee, V. M. (1997). Lithium reduces tau phosphorylation by inhibition of glycogen synthase kinase-3. *J. Biol. Chem.* **272**, 25326-25332.

Huang, K. X. and Paudel, H. K. (2000). Ser67-phosphorylated inhibitor 1 is a potent protein phosphatase 1 inhibitor. *Proc. Natl. Acad. Sci. USA* **97**, 5824-5829.

Hughes, K., Nikolakaki, E., Plyte, S. E., Totty, N. F. and Woodgett, J. R. (1993). Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *EMBO J.* **12**, 803-808.

Hutton, M., Lendon, C. L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A. et al. (1998). Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* **393**, 702-705.

Iqbal, K., Grundke-Iqbal, I., Zaidi, T., Merz, P. A., Wen, G. Y., Shaikh, S. S., Wisniewski, H. M., Alafuzoff, I. and Winblad, B. (1986). Defective brain microtubule assembly in Alzheimer's disease. *Lancet* **2**, 421-426.

Iqbal, K., Alonso-Adel, C., El-Akkad, E., Gong, C. X., Haque, N., Khatoon, S., Pei, J. J., Tsujio, I., Wang, J. Z. and Grundke-Iqbal, I. (2002). Significance and mechanism of Alzheimer neurofibrillary degeneration and therapeutic targets to inhibit this lesion. *J. Mol. Neurosci.* **19**, 95-99.

Ishiguro, K., Shiratsuchi, A., Sato, S., Omori, A., Arioka, M., Kobayashi, S., Uchida, T. and Imahori, K. (1993). Glycogen synthase kinase 3 beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. *FEBS Lett.* **325**, 167-172.

Ishihara, T., Hong, M., Zhang, B., Nakagawa, Y., Lee, M. K., Trojanowski, J. Q. and Lee, V. M. (1999). Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* **24**, 751-762.

Jameson, L., Frey, T., Zeeberg, B., Dalldorf, F. and Caplow, M. (1980). Inhibition of microtubule assembly by phosphorylation of microtubule-associated proteins. *Biochemistry* **19**, 2472-2479.

Johnson, G. V. and Bailey, C. D. (2002). Tau, where are we now? *J. Alzheimers Dis.* **4**, 375-398.

Johnson, G. V. and Jenkins, S. M. (1999). Tau protein in normal and Alzheimer's disease brain. *J. Alzheimers Dis.* **1**, 307-328.

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

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

Kerokoski, P., Suuronen, T., Salminen, A., Soininen, H. and Pirttila, T. (2002). Cleavage of the cyclin-dependent kinase 5 activator p35 to p25 does not induce tau hyperphosphorylation. *Biochem. Biophys. Res. Commun.* **298**, 693-698.

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

Kosik, K. S., Orecchio, L. D., Bakalis, S. and Neve, R. L. (1989). Developmentally regulated expression of specific tau sequences. *Neuron* **2**, 1389-1397.

Kusakawa, G., Saito, T., Onuki, R., Ishiguro, K., Kishimoto, T. and Hisanaga, S. (2000). Calpain-dependent proteolytic cleavage of the p35 cyclin-dependent kinase 5 activator to p25. *J. Biol. Chem.* **275**, 17166-17172.

Lee, V. M., Goedert, M. and Trojanowski, J. Q. (2001). Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* **24**, 1121-1159.

Leroy, K. and Brion, J. P. (1999). Developmental expression and localization of glycogen synthase kinase-3beta in rat brain. *J. Chem. Neuroanat.* **16**, 279-293.

Liao, H., Li, Y., Brautigan, D. L. and Gundersen, G. G. (1998). Protein phosphatase 1 is targeted to microtubules by the microtubule-associated protein Tau. *J. Biol. Chem.* **273**, 21901-21908.

Lindwall, G. and Cole, R. D. (1984). Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J. Biol. Chem.* **259**, 5301-5305.

Litersky, J. M., Johnson, G. V., Jakes, R., Goedert, M., Lee, M. and Seubert, P. (1996). Tau protein is phosphorylated by cyclic AMP-dependent protein kinase and calcium/calmodulin-dependent protein kinase II within its microtubule-binding domains at Ser-262 and Ser-356. *Biochem. J.* **316**, 655-660.

Liu, F., Iqbal, K., Grundke-Iqbal, I. and Gong, C. X. (2002). Involvement of aberrant glycosylation in phosphorylation of tau by cdk5 and GSK-3beta. *FEBS Lett.* **530**, 209-214.

Lovestone, S., Hartley, C. L., Pearce, J. and Anderton, B. H. (1996). Phosphorylation of tau by glycogen synthase kinase-3 beta in intact mammalian cells: the effects on the organization and stability of microtubules. *Neuroscience* **73**, 1145-1157.

Lovestone, S., Davis, D. R., Webster, M. T., Kaech, S., Brion, J. P., Matus, A. and Anderton, B. H. (1999). Lithium reduces tau phosphorylation: effects in living cells and in neurons at therapeutic concentrations. *Biol. Psychiatry* **45**, 995-1003.

Lu, Q. and Wood, J. G. (1993). Functional studies of Alzheimer's disease tau protein. *J. Neurosci.* **13**, 508-515.

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

Lund, E. T., McKenna, R., Evans, D. B., Sharma, S. K. and Mathews, W. R. (2001). Characterization of the in vitro phosphorylation of human tau by tau protein kinase II (cdk5/p20) using mass spectrometry. *J. Neurochem.* **76**, 1221-1232.

Mandelkow, E. M., Stamer, K., Vogel, R., Thies, E. and Mandelkow, E. (2003). Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. *Neurobiol. Aging* **24**, 1079-1085.

Mandelkow, E. M., Thies, E., Trinczek, B., Biernat, J. and Mandelkow, E. (2004). MARK/PAR-1 kinase is a regulator of microtubule dependent transport in axons. *J. Cell Biol.* **167**, 99-110.

Mandell, J. W. and Banker, G. A. (1996). A spatial gradient of tau protein phosphorylation in nascent axons. *J. Neurosci.* **16**, 5727-5740.

Morfini, G., Szebenyi, G., Brown, H., Pant, H. C., Pigino, G., DeBoer, S., Beffert, U. and Brady, S. T. (2004). A novel CDK5-dependent pathway for regulating GSK3 activity and kinesin-driven motility in neurons. *EMBO J.* **23**, 2235-2245.

Nishimura, I., Yang, Y. and Lu, B. (2004). PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in *Drosophila*. *Cell* **116**, 671-682.

Noble, W., Olm, V., Takata, K., Casey, E., Mary, O., Meyerson, J., Gaynor, K., LaFrancois, J., Wang, L., Kondo, T. et al. (2003). Cdk5 is a key factor in tau aggregation and tangle formation in vivo. *Neuron* **38**, 555-565.

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

Patrick, G. N., Zhou, P., Kwon, Y. T., Howley, P. M. and Tsai, L. H. (1998). p35, the neuronal-specific activator of cyclin-dependent kinase 5 (Cdk5) is degraded by the ubiquitin-proteasome pathway. *J. Biol. Chem.* **273**, 24057-24064.

Patrick, G. N., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P. and Tsai, L. H. (1999). Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* **402**, 615-622.

Patzke, H. and Tsai, L. H. (2002). Calpain-mediated cleavage of the cyclin-dependent kinase-5 activator p39 to p29. *J. Biol. Chem.* **277**, 8054-8060.

Perez, M., Hernandez, F., Lim, F., Diaz-Nido, J. and Avila, J. (2003). Chronic lithium treatment decreases mutant tau protein aggregation in a transgenic mouse model. *J. Alzheimers Dis.* **5**, 301-308.

Phiel, C. J., Wilson, C. A., Lee, V. M. and Klein, P. S. (2003). GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature* **423**, 435-439.

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

Probst, A., Gotz, J., Wiederhold, K. H., Tolnay, M., Mistl, C., Jaton, A. L., Hong, M., Ishihara, T., Lee, V. M., Trojanowski, J. Q. et al. (2000). Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. *Acta Neuropathol.* **99**, 469-481.

Rapoport, M., Dawson, H. N., Binder, L. I., Vitek, M. P. and Ferreira, A. (2002). Tau is essential to beta-amyloid-induced neurotoxicity. *Proc. Natl. Acad. Sci. USA* **99**, 6364-6369.

Rohn, T. T., Rissman, R. A., Davis, M. C., Kim, Y. E., Cotman, C. W. and Head, E. (2002). Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. *Neurobiol. Dis.* **11**, 341-354.

Sayas, C. L., Avila, J. and Wandosell, F. (2002). Regulation of neuronal cytoskeleton by lysophosphatidic acid: role of GSK-3. *Biochim. Biophys. Acta* **1582**, 144-153.

Schneider, A., Biernat, J., von Bergen, M., Mandelkow, E. and Mandelkow, E. M. (1999). Phosphorylation that detaches tau protein from microtubules (Ser262, Ser214) also protects it against aggregation into Alzheimer paired helical filaments. *Biochemistry* **38**, 3549-3558.

Scott, C. W., Spreen, R. C., Herman, J. L., Chow, F. P., Davison, M. D., Young, J. and Caputo, C. B. (1993). Phosphorylation of recombinant tau by cAMP-dependent protein kinase. Identification of phosphorylation sites and effect on microtubule assembly. *J. Biol. Chem.* **268**, 1166-1173.

Seitz, A., Kojima, H., Oiwa, K., Mandelkow, E. M., Song, Y. H. and Mandelkow, E. (2002). Single-molecule investigation of the interference between kinesin, tau and MAP2c. *EMBO J.* **21**, 4896-4905.

Seubert, P., Mawal-Dewan, M., Barbour, R., Jakes, R., Goedert, M., Johnson, G. V., Litersky, J. M., Schenk, D., Lieberburg, I., Trojanowski, J. Q. et al. (1995). Detection of phosphorylated Ser262 in fetal tau, adult tau, and paired helical filament tau. *J. Biol. Chem.* **270**, 18917-18922.

Shahani, N. and Brandt, R. (2002). Functions and malfunctions of the tau proteins. *Cell. Mol. Life Sci.* **59**, 1668-1680.

Shelton, S. B. and Johnson, G. V. (2004). Cyclin-dependent kinase-5 in neurodegeneration. *J. Neurochem.* **88**, 1313-1326.

Shelton, S. B., Krishnamurthy, P. and Johnson, G. V. (2004). Effects of cyclin-dependent kinase-5 activity on apoptosis and tau phosphorylation in immortalized mouse brain cortical cells. *J. Neurosci. Res.* **76**, 110-120.

Sironi, J. J., Yen, S. H., Gondal, J. A., Wu, Q., Grundke-Iqbal, I. and Iqbal, K. (1998). Ser-262 in human recombinant tau protein is a markedly more favorable site for phosphorylation by CaMKII than PKA or PhK. *FEBS Lett.* **436**, 471-475.

Smith, D. S., Greer, P. L. and Tsai, L. H. (2001). Cdk5 on the brain. *Cell Growth Differ.* **12**, 277-283.

Spillantini, M. G., Murrell, J. R., Goedert, M., Farlow, M. R., Klug, A. and Ghetti, B. (1998). Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. USA* **95**, 7737-7741.

Spittaels, K., van den Haute, C., van Dorpe, J., Bruynseels, K., Vandezande, K., Laenen, I., Geerts, H., Mercken, M., Sciot, R., van Lommel, A. et al. (1999). Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein. *Am. J. Pathol.* **155**, 2153-2165.

Spittaels, K., van den Haute, C., van Dorpe, J., Geerts, H., Mercken, M., Bruynseels, K., Lasrado, R., Vandezande, K., Laenen, I., Boon, T. et al. (2000). 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**, 41340-41349.

Stambolic, V., Ruel, L. and Woodgett, J. R. (1996). Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr. Biol.* **6**, 1664-1668.

Stamer, K., Vogel, R., Thies, E., Mandelkow, E. and Mandelkow, E. M. (2002). Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J. Cell Biol.* **156**, 1051-1063.

Stoothoff, W. H. and Johnson, G. V. (2004). Tau phosphorylation: physiological and pathological consequences. *Biochim. Biophys. Acta* (in press).

Su, Y., Ryder, J., Li, B., Wu, X., Fox, N., Solenberg, P., Brune, K., Paul, S., Zhou, Y., Liu, F. et al. (2004). Lithium, a common drug for bipolar disorder treatment, regulates amyloid-beta precursor protein processing. *Biochemistry* **43**, 6899-6908.

Sutherland, C., Leighton, I. A. and Cohen, P. (1993). Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. *Biochem. J.* **296**, 15-19.

Takashima, A., Noguchi, K., Sato, K., Hoshino, T. and Imahori, K. (1993). Tau protein kinase I is essential for amyloid beta-protein-induced neurotoxicity. *Proc. Natl. Acad. Sci. USA* **90**, 7789-7793.

Takashima, A., Noguchi, K., Michel, G., Mercken, M., Hoshi, M., Ishiguro, K. and Imahori, K. (1996). Exposure of rat hippocampal neurons to amyloid beta peptide (25-35) induces the inactivation of phosphatidyl inositol-3 kinase and the activation of tau protein kinase I/glycogen synthase kinase-3 beta. *Neurosci. Lett.* **203**, 33-36.

Takei, Y., Teng, J., Harada, A. and Hirokawa, N. (2000). Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. *J. Cell Biol.* **150**, 989-1000.

Tatebayashi, Y., Haque, N., Tung, Y. C., Iqbal, K. and Grundke-Iqbal, I. (2004). Role of tau phosphorylation by glycogen synthase kinase-3beta in the regulation of organelle transport. *J. Cell Sci.* **117**, 1653-1663.

Van den Haute, C., Spittaels, K., van Dorpe, J., Lasrado, R., Vandezande, K., Laenen, I., Geerts, H. and van Leuven, F. (2001). Coexpression of human cdk5 and its activator p35 with human protein tau in neurons in brain of triple transgenic mice. *Neurobiol. Dis.* **8**, 32-44.

Wagner, U., Utton, M., Gallo, J. M. and Miller, C. C. (1996). Cellular phosphorylation of tau by GSK-3 beta influences tau binding to microtubules and microtubule organisation. *J. Cell Sci.* **109**, 1537-1543.

Watanabe, A., Hasegawa, M., Suzuki, M., Takio, K., Morishima-Kawashima, M., Titani, K., Arai, T., Kosik, K. S. and Ihara, Y. (1993). In vivo phosphorylation sites in fetal and adult rat tau. *J. Biol. Chem.* **268**, 25712-25717.

Weingarten, M. D., Lockwood, A. H., Hwo, S. Y. and Kirschner, M. W. (1975). A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. USA* **72**, 1858-1862.

Wood, J. G., Mirra, S. S., Pollock, N. J. and Binder, L. I. (1986). Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau). *Proc. Natl. Acad. Sci. USA* **83**, 4040-4043.

Woodgett, J. R. (1990). Molecular cloning and expression of glycogen synthase kinase-3/factor A. *EMBO J.* **9**, 2431-2438.

Yoshida, H. and Ihara, Y. (1993). Tau in paired helical filaments is functionally distinct from fetal tau: assembly incompetence of paired helical filament-tau. *J. Neurochem.* **61**, 1183-1186.