Functional implications of the association of tau with the plasma membrane

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Abstract
Tau is an abundant microtubule-associated protein which regulates the stability of the cytoskeleton. Tau binds microtubules directly through microtubule-binding domains in its C-terminus. However, tau is not only located in the cytosol of cells, but also associated with other intracellular domains, including the plasma membrane, suggesting that tau may have additional functions other than stabilizing the neuronal cytoskeleton. Localization of tau at the cell surface appears to be dependent on interactions of the N-terminal projection domain of tau. Furthermore, membrane-associated tau is dephosphorylated at serine/threonine residues, suggesting that the phosphorylation state of tau regulates its intracellular trafficking. Dephosphorylation of tau may increase the association of tau with trafficking proteins which target tau to the plasma membrane. Thus it is possible that the hyperphosphorylation of tau may contribute to the pathogenesis of Alzheimer’s disease by promoting the formation of neurofibrillary tangles from cytosolic tau, and also by inhibiting additional tau functions through disruption of its targeting to the plasma membrane.

Domain structure of tau
Tau is an axonally enriched microtubule-binding protein involved in stabilization of the neuronal cytoskeleton [1]. Tau binds directly to microtubules via its microtubule-binding repeat domain, which is located at the C-terminal end of the protein and is encoded by exons 9–12 [2]. Alternative splicing of exon 10 results in two different sets of tau isoforms: one set containing three tubulin-binding repeats (3R tau), and a second set containing four repeats (4R tau) [3]. The inclusion of exon 10 is developmentally regulated: 3R tau is expressed early in development and in adulthood, whereas the 4R tau isoform is expressed only in adult brain [4]. Since the 3R isoform of tau binds less well to microtubules than does 4R tau, it is possible that 3R tau may enable the required increased plasticity of the nervous system during development [5]. In the adult brain, the ratio of 4R to 3R tau expression is approximately equal; however, alterations of this ratio, particularly increased 4R tau expression, are characteristic of several tauopathies [6]. The N-terminal region of tau does not interact directly with microtubules and it contains the projection domain. Alternative splicing of exons 2 and 3 regulates the inclusion of none, one or two negatively charged 29 amino acid inserts into this N-terminal region, which, together with the splicing of exon 10, produces a total of six distinct tau isoforms in the adult central nervous system [3]. The projection domain of tau also includes a highly charged proline-rich domain towards the central region of the tau molecule and lying C-terminal to the inserts encoded by exons 2 and 3 [7]. To date, however, the function of the projection domain is not well understood. However, through its proline-rich domain, it interacts with other microtubule-associated proteins, including actin [8]. Other potential functions of this region include regulation of cell signalling through binding to the SH3 (Src homology 3) domain of plasma-membrane-associated proteins such as Src family kinases [9] and phospholipase Cγ [10].

Association of tau with the plasma membrane is regulated by its projection domain
In addition to the presence of tau in the cytosolic compartment, where it interacts with microtubules, accumulating evidence suggests that tau is also targeted to several intracellular membranes, including the endoplasmic reticulum [11], the Golgi [12] and the plasma membrane [13]. Although it is not known exactly how tau interacts with these membranes, evidence suggests that the projection domain may be involved in this process, especially in localizing tau to the cell surface. In rat PC12 phaeochromocytoma cells stably overexpressing tau, imaging of saponin-extracted cells showed a co-localization between tau and the plasma membrane [13]. In this study, when a cDNA construct containing only the N-terminal tau projection domain was expressed in PC12 cells, diffuse labelling was observed, since this construct lacked the C-terminal domain that mediates microtubule binding. However, following extraction of cytosolic proteins, both confocal and electron microscopy revealed that this N-terminal tau construct was closely associated with the inner...
Phosphorylation of tau modulates its association with membranes

The cellular localization of tau could be dependent upon its phosphorylation state. Although tau undergoes a variety of post-translational modifications, its phosphorylation has been the most thoroughly investigated, since hyperphosphorylation of tau occurs during the pathogenesis of AD (Alzheimer’s disease) and other tauopathies. Abnormally phosphorylated tau accumulates in PHFs (paired helical filaments) which comprise the main component of the characteristic intracellular tangles found in brains affected by AD [15]. In healthy brains, phosphorylation of tau is developmentally and functionally regulated, with a high level of phosphorylation evident at early developmental stages, possibly related to increased plasticity, and relatively decreased phosphorylation in adulthood. Thus the interaction of tau with microtubules is regulated by phosphorylation at specific residues on tau, including Ser214, Ser262 and Thr231 [16-18]. Several protein kinases, including GSK3β (glycogen synthase kinase 3β), Cdk5 (cyclin-dependent kinase 5) and CK1 (casein kinase 1), phosphorylate tau at serine/threonine sites [19-22]. Dephosphorylation of tau is accomplished by PP1 (protein phosphatases), primarily PP2A and PP2B [23,24]. The phosphorylation state of tau is therefore a tightly regulated process involving many different kinases and phosphatases.

In neurons, phosphorylation regulates the intracellular trafficking and plasma membrane association of many proteins. For example, the voltage-gated potassium channel Kv4.2 is trafficked to the cell surface following phosphorylation on a single serine residue by protein kinase A [25]. Delivery of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors to the plasma membrane is also regulated by phosphorylation; stimulation of NMDA (N-methyl-d-aspartate) receptors leads to the serine phosphorylation of the AMPA subunit GluR (glutamate receptor) 1, a modification necessary for its subsequent trafficking to extrasynaptic sites [26]. Moreover, dephosphorylation of GluR1 at this serine residue stimulates the removal of the subunit from the plasma membrane [26]. Furthermore, protein kinase C phosphorylation of the kainate receptor subunit GluR6 on two C-terminal serine residues significantly enhances expression of the receptor at the cell surface [27]. Phosphorylation of specific residues is therefore critically important to regulate the trafficking of a variety of different proteins to the neuronal plasma membrane.

The association of tau with the plasma membrane also appears to be dependent upon its phosphorylation state. Plasma membrane-associated tau is dephosphorylated at the Tau-1 antibody epitope (Ser199/Ser202) in SH-SY5Y neuroblastoma cells [14,28] and in PC12 cells transfected with tau [29]. Furthermore, exogenously expressed tau that is phosphorylated at the PHF-1 antibody epitope (Ser396/Ser404) or the AT8 epitope (Ser199/Ser202) is not associated with the cell membrane in PC12 cells [29]. Interestingly, in this work, the plasma membrane preparations are reportedly free of tubulin, indicating that the interaction of tau with the cell surface is independent of its interaction with microtubules [29]. These results indicate that phosphorylation, in addition to modulating the association of tau with microtubules, might also regulate trafficking of tau to the plasma membrane. Furthermore, these studies suggest that, during the development of AD, hyperphosphorylation might disrupt tau function by inhibiting its association with the plasma membrane.

Tyrosine kinases and intracellular targeting of proteins

Tau has also been reported to be present within cell-surface lipid-rich microdomains of the plasma membrane [30]. Evidence suggests that the amount of tau associated with these microdomains might be regulated by tyrosine phosphorylation. For example, treatment of hippocampal neurons with soluble oligomers of Aβ (amyloid β-peptide), a component of the amyloid plaques that characterize AD-affected brain, led to increased amounts of tau in lipid-rich microdomains, while concurrently increasing the tyrosine phosphorylation of tau [31]. Several tyrosine kinases, including Fyn, Src, Lck and c-Abl, are reported to phosphorylate tau on one or more of its five tyrosine residues [32-34]. Tau interacts directly with the SH3 domains of these kinases through a Pro-Xaa-Xaa-Pro motif contained within its proline-rich domain [9]. Interestingly, binding of tau to the kinase SH3 domains may be regulated by the serine/threonine phosphorylation state of tau. For example, the highly phosphorylated PHF tau isolated from AD-affected brain does not bind to the Fyn SH3 domain [10].

Interactions between tau and tyrosine kinases such as Fyn might regulate the intracellular localization of tau. Fyn is targeted to the plasma membrane by palmitoylation [35] and is involved in protein trafficking to the plasma membrane [36]. In transfected COS-7 cells, expression of Fyn increases the surface expression of amyloid precursor protein through phosphorylation on tyrosine residues [37]. Fyn has also been shown to stabilize the NMDA receptor at the plasma membrane in transfected neurons; phosphorylation of a tyrosine residue in the NR2B subunit by Fyn prevents clathrin-mediated endocytosis of this receptor [38]. Thus it is possible that targeting of tau to the cell surface could occur in a similar fashion, with increased Fyn activity potentially inducing increased plasma membrane expression of tau, although this has yet to be proven. Furthermore, dephosphorylation of tau may promote its association with the SH3 domain of a tyrosine kinase, which then targets tau to the cell surface. Alternatively, dephosphorylation of tau at serine/threonine sites may enable a tyrosine kinase to then phosphorylate...
tau on specific tyrosine sites, promoting the trafficking of tau to the plasma membrane by another mechanism. This hypothesis is supported by the finding that plasma membrane-associated tau is largely dephosphorylated at several serine/threonine sites [14,28]. Accumulating evidence therefore suggests that targeting of tau to the plasma membrane may be regulated by the interaction of the tau N-terminal projection domain with both the plasma membrane and the SH3 domain of tyrosine kinases such as Fyn.

**Association of tau with the membrane: relevance to neurodegeneration**

The targeting of tau to the plasma membrane and to lipid-rich membrane microdomains suggests novel functions of tau, such as participation in intracellular signalling pathways [39,40]. Possible interactions between tau and cell signalling are of particular interest, since disruption of signalling pathways and loss of synapses occur early in the pathogenesis of AD [41,42]. AD induces changes in several neurotransmitter receptor systems, by decreasing the expression of receptors such as NMDA and AMPA [43], as well as by disrupting signalling involving receptors such as the M₁ muscarinic acetylcholine receptor [44]. At the cell surface, tau could thus interact with proteins involved in synaptic signalling. For example, it has been reported that tau binds directly to the GluR2/3 subunits of the AMPA receptor in the rat hippocampus [45]. Once associated with the plasma membrane, tau might also be secreted into extracellular space and participate in cell–cell signalling. Although it has long been assumed that the presence of extracellular tau is the result of neuronal death, a recent report in lamprey neurons overexpressing human tau shows that the secretion of tau from neurons is a regulated process, dependent upon phosphorylation of tau by Fyn and requiring the N-terminal projection domain of tau [46]. Indeed, extracellular tau can activate the muscarinic acetylcholine receptors M₁ and M₃ and stimulate intracellular Ca²⁺ release in neurons [47]. Importantly, the affinity of tau for these receptors is greater than that of acetylcholine [48]. Therefore, in addition to affecting regulation of cytoskeletal dynamics directly, sequestration of tau into tangles might also reduce the amount of membrane-bound tau and thus disrupt functions related to this association. For example, the formation of intracellular tau aggregates correlates with hippocampal synaptic dys-function in mice overexpressing human tau [49]. Targeting of tau to the plasma membrane might also play a role in neuronal development, since tau interacts with the membrane in distal axons during the development of neuronal polarity [13,50]. However, further investigation is necessary to clarify the function of tau at the plasma membrane, and how disruption of this function may play a role in the development of AD.

Tau is localized to many different regions within the cell, including the plasma membrane which may confer novel functions for tau in addition to its role in stabilization of microtubules. Targeting of tau to specific intracellular domains appears to be regulated by its phosphorylation state, which is determined by the activity of many different tau kinases and phosphatases. Dephosphorylation of tau may promote its association with other proteins, such as a tyrosine kinase, which enable the targeting of tau to the plasma membrane. In diseases such as AD, it is therefore possible that tau hyperphosphorylation might lead to neuronal dysfunction in several ways, by promoting the formation of neurofibrillary tangles from cytosolic tau, and by inhibiting important signalling functions of tau by preventing its association with the neuronal plasma membrane.

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**References**


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