

# Role of Tau Protein in Both Physiological and Pathological Conditions

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|  |     |
|--|-----|
| I. Introduction  | 362 |
| II. The Tau Gene   | 362 |
| A. Human tau gene polymorphism   | 362 |
| B. Human tau gene expression   | 362 |
| III. Saithoin  | 363 |
| IV. Tau Protein  | 364 |
| A. Regions, domains, and motifs  | 364 |
| V. Cellular and Subcellular Localization of Tau Protein  | 365 |
| VI. Posttranslational Modifications of Tau   | 365 |
| A. Tau phosphorylation   | 365 |
| B. Tau glycosylation   | 367 |
| C. Tau ubiquitinylation  | 367 |
| D. Tau glycation   | 367 |
| E. Tau truncation and deamidation  | 367 |
| F. Tau oxidation   | 367 |
| G. Other modifications of tau  | 367 |
| VII. Tau Turnover  | 367 |
| VIII. Tau-Associated Proteins  | 367 |
| A. Binding of tau to chaperones  | 368 |
| IX. Tau Function   | 368 |
| X. Tau Pathology   | 368 |
| XI. Tau Assembly In Vitro  | 370 |
| A. Phosphorylation before or after assembly  | 370 |
| B. Toxic effects of modified tau   | 370 |
| C. PHF morphology  | 371 |
| XII. Tauopathies   | 371 |
| A. AD  | 371 |
| B. Corticobasal degeneration   | 371 |
| C. Downs' syndrome   | 371 |
| D. Frontotemporal dementia with parkinsonism linked to chromosome 17   | 371 |
| E. Pick's disease  | 372 |
| F. Postencephalic parkinsonism   | 372 |
| G. Progressive supranuclear palsy  | 372 |
| H. Niemann-Pick type C disease   | 372 |
| I. Other tauopathies   | 372 |
| J. Lack of pathology in some neurons   | 372 |
| XIII. Animal Models  | 372 |
| A. Lower eukaryotes: <i>Drosophila</i> to lamprey  | 372 |
| B. Vertebrates   | 373 |
| XIV. A Working Hypothesis to Explain Tau Pathology Due to Tau Hyperphosphorylation or Tau Filament Formation | 375 |
| XV. Summary  | 376 |

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**Avila, Jesús, José J. Lucas, Mar Pérez, and Félix Hernández.** Role of Tau Protein in Both Physiological and Pathological Conditions. *Physiol Rev* 84: 361–384, 2004; 10.1152/physrev.00024.2003.—The morphology of a neuron is determined by its cytoskeletal scaffolding. Thus proteins that associate with the principal cytoskeletal compo-

nents such as the microtubules have a strong influence on both the morphology and physiology of neurons. Tau is a microtubule-associated protein that stabilizes neuronal microtubules under normal physiological conditions. However, in certain pathological situations, tau protein may undergo modifications, mainly through phosphorylation, that can result in the generation of aberrant aggregates that are toxic to neurons. This process occurs in a number of neurological disorders collectively known as tauopathies, the most commonly recognized of which is Alzheimer's disease. The purpose of this review is to define the role of tau protein under normal physiological conditions and to highlight the role of the protein in different tauopathies.

## I. INTRODUCTION

Neurons are cells with a very complex morphology that develop two types of cytoplasmic extensions, axons and dendrites. Neural transmission occurs through these processes, and therefore, any changes in neuronal morphology may affect their behavior and even produce pathological events. Indeed, it should be born in mind that the morphological differentiation of a neuron involves the extensive rearrangement of the cytoskeleton, which is responsible for maintaining the cell's shape.

The cytoskeleton is composed of three main components: the microtubules, the microfilaments, and the intermediate filaments. Microtubules are very dynamic structures, and in proliferating cells such as neuroblasts (neuron precursors), their probability of assembly is the same as that of depolymerization in all directions. This equilibrium results in the cell maintaining a spherelike morphology. However, during the differentiation of a neuroblast into a neuron (209), the microtubules become stabilized in specific directions, thereby generating the cytoplasmic extensions that will become the axon and the dendrites (209).

It has been suggested that specific proteins may serve to stabilize microtubules and such proteins including the microtubule-associated proteins (or MAPs) MAP1A, MAP1B, MAP2, and tau (Fig. 1B). In support of this hypothesis, an asymmetric distribution of MAPs (205) is seen in mature neurons (52), and tau is preferentially localized in axons (24). Indeed, as well as being present mainly in the axon of a neuron, tau function and dysfunction have been related to axonal microtubule function, both alone and in synergy with other MAPs (101). Furthermore, in pathological situations, tau has additionally been shown to be capable of forming aberrant fibrillar polymers (Fig. 1C).

Tau protein was discovered almost simultaneously in the United States and Europe as a protein that lowered the concentration at which tubulin polymerizes into microtubules in the brain (45, 46, 74, 291). At the same time, other high-molecular-weight MAPs were also found to influence the cycles of microtubule assembly and disassembly in vitro (255). As a result, the question rose as to whether tau was simply a degradation product of the high-molecular-weight MAPs. The groups of Kirschner

(45) and Nuñez (74) rapidly showed that tau was indeed an independent protein.

Several good reviews dealing with specific features of tau have been published recently (32, 147, 160, 182, 254). Thus here we intend to cover the more general aspects of this protein, to discuss recent developments, and to highlight what we believe may be the future for the study of tau.

## II. THE TAU GENE

A cDNA for tau was first isolated from a mouse brain expression library (177), and subsequently, it was cloned from other species including goat (222), chicken (307), bovine (133), and human (91, 93, 94). More recently, tau sequences have been described in a number of distinct species (222).

### A. Human Tau Gene Polymorphism

Two different tau gene haplotypes have been identified (H1 and H2), consisting of eight common single nucleotide polymorphisms. H1 is the most common, and it is overexpressed in disorders like progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD; Refs. 60, 140, 229). In addition, a polymorphic dinucleotide repeat has been also identified in intron 9 (21).

### B. Human Tau Gene Expression

The human gene is located on chromosome 17 (223), where it occupies over 100 kb and contains at least 16 exons (10; Fig. 2). Following a GC-rich 5'-region, a single untranslated exon exists (exon-1; Ref. 11). Upstream of this exon there are several DNA sequences that contain consensus binding sites for promiscuous transcription factors such as AP2 or SP1. Tau is mainly expressed in neurons, and an interaction with a neural specific factor has been proposed (246, 247). Nevertheless, the neural specific expression of the protein could be also due to the presence of possible silencer elements in nonneural cells (172).

The tau gene is transcribed into nuclear RNA that, by alternative splicing, yields different mRNA species. The translation of these distinct spliced mRNAs results in the

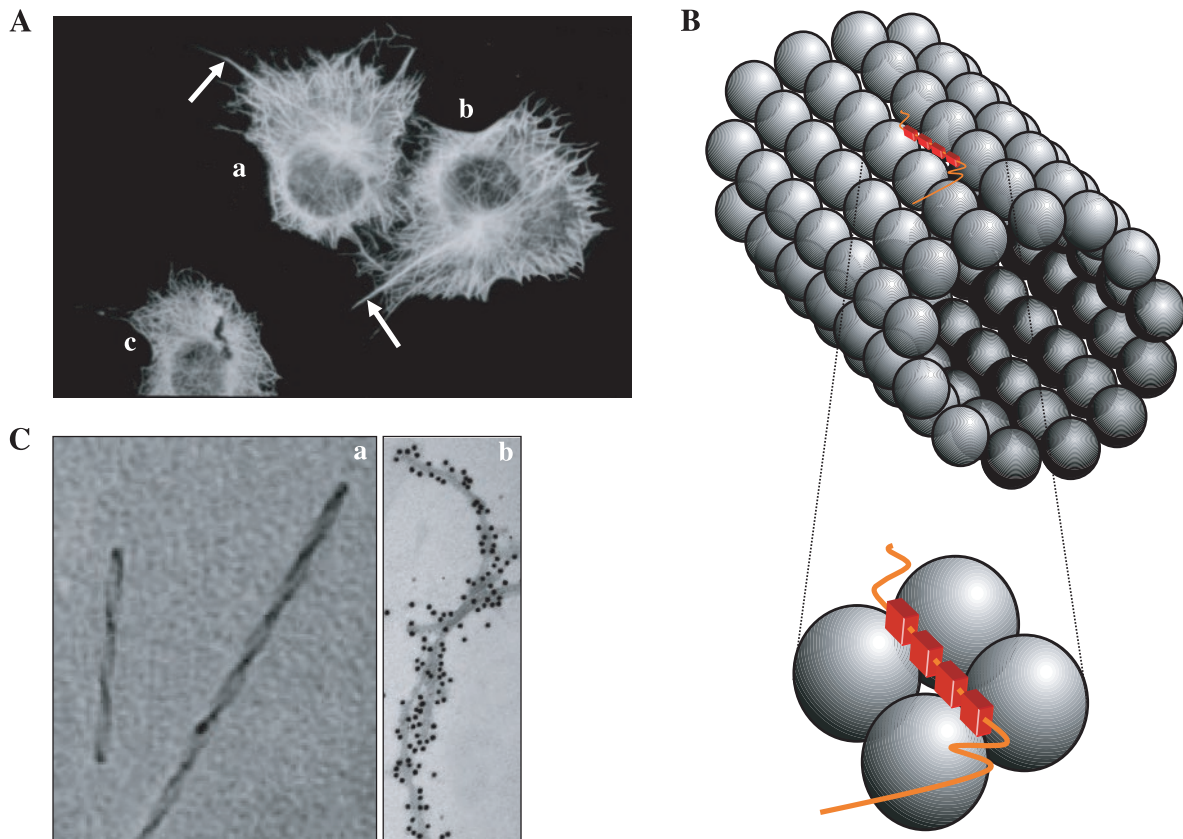


FIG. 1. Tau is a microtubule-associated protein that stabilizes microtubules and that is able to self-aggregate in pathological conditions. *A*: the ectopic expression of tau in nonneural cells (*a* and *b*) promotes the stabilization of microtubules, leading to the formation of cytoplasmic extensions (arrows) that are not normally seen in those cells that are not expressing tau (*c*). *B*: scheme showing tau as a microtubule-associated protein (the size of the tau molecule is exaggerated compared with that of the microtubule). A view of the binding of tau to a tubulin dimer is indicated. *C*: in pathological situations (like Alzheimer's disease), the tau protein can form aberrant filaments (*a*). These filaments can bind to antibodies raised against the tau protein (*b*).

production of the different tau isoforms. The expression of different tau isoforms with differing numbers of exons is characteristic during brain development. Indeed, isoforms lacking exon 10 are found at early developmental stages, or in specific cell types like granular cells of dentate gyrus (94). The determinants of exon 10 splicing have been studied in detail (67, 68), and this splicing seems to be regulated by the phosphorylation of splicing factors (119) such as the SC35-like protein, which may play a role in this regulation (F. Hernández, M. Pérez, J. J. Lucas, and J. Avila, unpublished observations). Other tau isoforms that lack exon 2, or exons 2 and 3 (133), have also been described. Indeed, exons 2, 3, and 10 are alternatively spliced and are adult brain specific. Exons 2 and 3 are alternatively spliced cassettes; exon 2 can appear alone, but exon 3 never appears independently of exon 2 (9). Furthermore, in humans there is little (if any) expression of exons 6 and 8.

In the peripheral nervous system (PNS), there is a high-molecular-weight tau isoform expressing the exon

4A, which yields a protein known as big tau with an approximate size of 100 kDa (51, 92, 225). In the central nervous system, alternative splicing of exons 2, 3, and 10 results in the appearance of six tau isoforms. Because exon 10 encodes for one of the regions involved in the binding of tau to microtubules (see below), alternative splicing of exon 10 produces tau isoforms, with either three (tau 3R without exon 10) or four (tau 4R with exon 10) tubulin/microtubule binding regions. In chicken, an extra tubulin binding region appears (tau 5R) (307).

### III. SAITHOIN

The entire intron-exon structure of tau was first described for bovine tau (132, 133), before that of the human gene (93). Recently, it was found that in intron 9 of human tau, there is a region that behaves like an exon, giving rise to the expression of a novel protein known as saithoin (48). Little is known about this protein that is nested

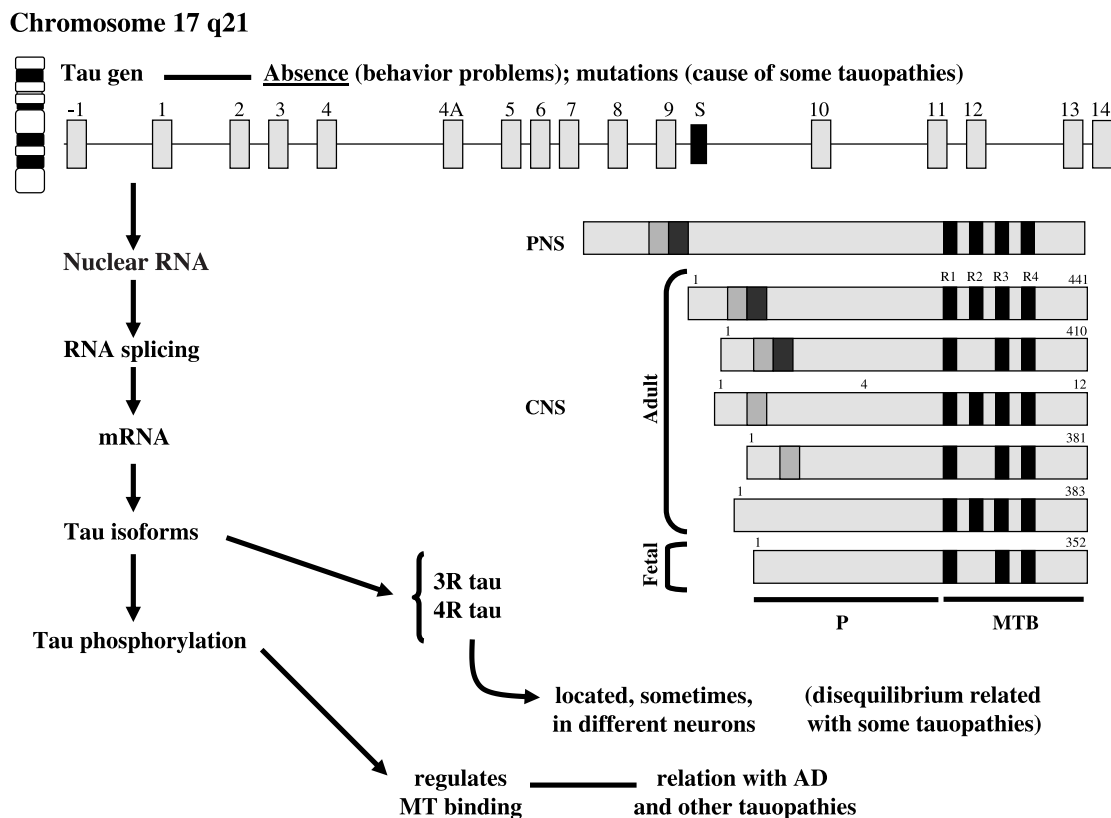


FIG. 2. Tau expression. A single tau gene located on the human chromosome 17 is transcribed into the corresponding nuclear RNA that, after alternative splicing, yields several tau mRNAs. These mRNAs, upon translation, originate the different tau isoforms. The complexity of the tau isoforms can then be increased by posttranslational modification, such as phosphorylation. Changes in the tau gene, in the regulation of RNA splicing, or in the degree of tau phosphorylation can underlie the neurological diseases known as tauopathies, the best known of which is Alzheimer's disease. S indicates the existence of a DNA sequence encoding the protein saithoin in the intron between exons 9 and 10. A scheme of tau isoforms is also indicated, showing the projection (P) and the microtubule binding domains (MTB) on tau molecule. The tubulin binding repeats are also indicated in black within the tau molecule at the amino-terminal region exons 2 (gray) and 3 (dark gray) and, in the peripheral nervous system (PNS), exon 4A (dashed).

within the tau gene, although it has been suggested that a polymorphism in this gene involving the amino acid change, Q7R, is correlated with a higher incidence of Alzheimer's disease (AD) in patients (48; Fig. 1). This association was not corroborated in a subsequent study of late-onset AD (283), but nevertheless, an association between the QQ genotype and frontotemporal dementia was observed. Thus isoforms of saithoin could be linked to the onset of different tauopathies.

Saithoin has not yet been isolated and characterized; however, by analysis of its sequence it does appear to contain some similarities to certain nucleic acid binding proteins (48). Saithoin contains the regions SSYEESR and SLAWEV similar to those present in some transcription factors, and thus it may play a role in transcription or nucleic acid metabolism. Furthermore, it is still not clear whether saithoin is a protein that is only expressed in human cells and not in other organisms such as the mouse.

#### IV. TAU PROTEIN

Several different tau polypeptides appear after fractionation of a brain protein extract by gel electrophoresis. Some of these polypeptides are generated by alternative RNA splicing (94, 132, 133, 173) and others by post-translational modifications including phosphorylation (23, 76, 91).

Tau is a hydrophilic protein that has been widely characterized in solution where, by analysis of the circular dichroism spectra (46), it appears as a random coiled protein. With its sedimentation coefficient taken into account, tau has been suggested to be a highly asymmetric protein, compatible with the long rod structure observed by electron microscopy (134).

##### A. Regions, Domains, and Motifs

Brain tau isoforms have been divided into two large domains: the projection domain containing the amino-



terminal two-thirds of the molecule and the microtubule-binding domain containing the carboxy-terminal one-third of the molecule. In addition, the projection domain can be further divided into two regions: the amino-terminal region with a high proportion of acidic residues and the proline-rich region. The microtubule-binding domain has also been subdivided into the basic, true tubulin-binding region and the acidic carboxy-terminal region (Fig. 2).

Several distinct roles have been proposed for the projection domain including that of determining the spacing between axonal microtubules (40), interactions with other cytoskeletal proteins (134), or cation binding due to the presence of the acidic residues. Indeed, it has been proposed that an iron-binding motif exists in the tau protein (16). Other motifs that have been identified in this region include the KKXX sequence, involved in heparin binding (16), or the PPXXP or PXXP motifs in the proline-rich region that may play a role in the interaction of tau with proteins containing SH3 domains. As a result, this region may also play a role in the binding of tau to proteins associated with the plasma membrane (15, 16, 26). On the other hand, a role for exons 2 and 3 in the more efficient microtubule bundling has also been suggested (162).

The microtubule-binding domain contains three (tau 3R) or four (tau 4R) similar but not identical repetitive sequences of 31 or 32 residues. Each of these repeats can be divided in two parts, one composed of an 18 residue sequence that contains the minimal region with tubulin binding capacity, and the second, a less conserved domain of 13 (or 14) residues known as the inter repeat. It should be noted that in the tubulin binding regions, the sequence with the highest capacity to bind to microtubules is that contained within the first repeat, the following inter region, and the second repeat (103). The repeats bind to microtubules and can promote microtubule assembly (275). Tau has been reported to bind to microtubules in two ways. When binding was tested on previously assembled and closed microtubules, tau bound to the outer surface (2, 38) (Fig. 1B). However, when tau was mixed with tubulin and then assembled, tau binds to the inside of microtubules (163). As stated above, the alternative splicing of exon 10 may result in the expression of tau 3R or tau 4R in a cell, which in turn may produce some physiological differences in the cell. In fact, tau 4R binds microtubules with a greater affinity and can displace the previously bound tau 3R from microtubules (197).

Tau shares homologies with other proteins, the most important of which can be seen in the tubulin-binding region. This region is similar to sequences found in other MAPs, like MAP2 or MAP3/MAP4, which share a similar function. Additionally, short motifs within tau have also been found in other proteins. These include the tau sequence PGGGSVQIY from residues 301 to 310 [largest central nervous system (CNS) tau isoform] that can be

found in nucleoside phosphatases, residues 134–142, (TGSDDKKAK) in  $\beta$ -transductin or, in a less homologous way, the sequences in the pyruvate dehydrogenase  $\beta$ -subunit (VVSXXXS, residues 398–404) or type 2 adenosine receptors (YSSXXXS, residues 197–202). Finally, proteins related to tau have been found in lower eukaryotes like *Caenorhabditis elegans* (83), *Drosophila* (150, 285), goldfish (187), and bullfrog (305).

## V. CELLULAR AND SUBCELLULAR LOCALIZATION OF TAU PROTEIN

Tau is mainly a neuronal protein, although its presence in different types of glia cells has been also reported in some neural diseases (see, for example, Refs. 16, 41). In neural cells, tau can associate with the plasma membrane (15, 16, 19, 26), in an interaction that could be modulated by tau phosphorylation, or it can be associated with microtubules, as previously indicated. Also, the presence of a nuclear antigen reacting with many tau antibodies has been reported, mainly in proliferating cells (25, 109, 195). Before its transport to the nucleus, tau appears to be phosphorylated in the cytosol (109).

In developing neurons, the phosphorylation of tau also seems to influence its distribution. Tau that is phosphorylated in its proline-rich region is mainly present in the somatodendritic compartment, whereas when this region becomes dephosphorylated, it can be found principally in the distal region of the axon (62, 203). Additionally, tau phosphorylated in its carboxy-terminal domain is also found mainly in the distal axonal region (62).

## VI. POSTTRANSLATIONAL MODIFICATIONS OF TAU

Several modifications have been described for tau protein including phosphorylation, glycosylation, ubiquitinylation, deamidation, oxidation, nitration, cross-linking, or glycation. The most studied of these has been phosphorylation.

### A. Tau Phosphorylation

In the decade of the 1980s, tau was defined as a phosphoprotein in many different studies (23, 112, 144). While these studies centered on the serine/threonine phosphorylation of the tau protein, more recently some attention has been paid to its phosphorylation on tyrosine (294).

There are 79 putative serine or threonine phosphorylation sites on the longest CNS tau isoform, which contains 441 residues. There are even more in PNS tau, although its phosphorylation has not been widely studied. These sites have been divided into two main groups: those

that can be modified by proline-directed kinases like tau protein kinase I (glycogen synthase kinase 3, GSK3), tau protein kinase II (cdk5), MAP kinase (p38), JNK, and other stress kinases or cdc2 and those that could be modified by non-proline-directed kinases like protein kinase A (PKA), protein kinase C (PKC), calmodulin (CaM) kinase II, MARK kinases (23, 49, 65, 86, 117, 146, 199, 251), or CKII that modifies residues close to acidic residues mainly in exons 2 and 3 (49). In many cases, phosphorylation regulates the binding of tau to microtubules, or as indicated above, to the membrane (26). Thus phosphorylation appears to be the predominant way in which tau function can be regulated (see below).

### 1. Tau phosphorylation by GSK3

Although different kinases may modify tau, there is emerging evidence that GSK3 plays an important role in regulating tau phosphorylation under normal (physiological) and pathological (nonphysiological) conditions. Two types of GSK3 phosphorylation have been proposed: primed (following prior phosphorylation of the substrate by another kinase) or unprimed phosphorylation (75). Primed phosphorylation appears to occur at threonine-231 and affects microtubule binding, while unprimed phosphorylation can take place at serine-396 or -404 and does not appear to affect microtubule binding (43). Interestingly, these sites can be identified by the AT180 and PHF-1 antibodies, respectively (43).

### 2. Tau phosphatases

Several phosphatases like protein phosphatase (PP) 1, PP2A, PP2B (calcineurin), and PP2C (77, 84, 148, 266, 302) have been implicated in reversing the phosphorylation of tau. However, only PP1, PP2A, and PP2B (97, 100) have been shown to dephosphorylate abnormally hyperphosphorylated tau. Although PP2C can dephosphorylate

tau when it is phosphorylated by PKA in vitro, it is not capable of dephosphorylating the abnormally hyperphosphorylated tau isolated from AD brain tissue (96). It seems probable that PP2A is the phosphatase that acts on most phosphorylation sites (87, 98). PP2A is composed of three subunits, A, B, and C, and it is the B subunits that are mainly involved in substrate recognition. However, few B subunits that can bind to tau have been described (265). PP2A binds to tau through its tubulin binding region (89). Mutations in this region could decrease the capacity of PP2A to bind to tau and, as a consequence, produce an increase in tau phosphorylation, a feature that has been observed in some FTDP-17 patients bearing such mutations (89).

### 3. Tau phosphorylation and development

The phosphorylation of tau is developmentally regulated; it is higher in fetal neurons and decreases with age during the development. Furthermore, a huge increase in the phosphorylation of tau arises in pathological situations (tauopathies) (112, 196, 252). Interestingly, phosphorylation at different sites could take place in different tau isoforms (127; Fig. 3). This could be due to the different cellular localization or subcellular compartmentalization of the different tau isoforms, or the fact that different kinases or phosphatases can modulate tau phosphorylation in a different way. It has proven possible to identify the phosphorylation of specific tau isoforms by analyzing the electrophoretic mobility of these isoforms in the presence or absence of phosphatases (116). However, it must be born in mind that the binding of tau isoforms to other proteins (microtubules) or the membrane may mask residues, preventing their phosphorylation in nonpathological situations, or like tau itself, in pathological situations. On the other hand, hyperphosphorylated tau has been described in extracts of both normal and AD tissue (171; see also legend to Fig. 3).

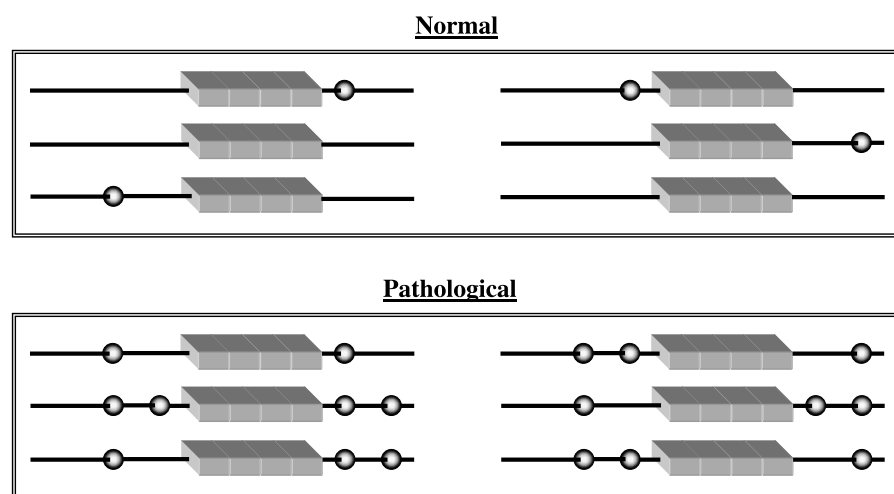


FIG. 3. Phosphorylation of tau isoforms. Different tau isoforms are phosphorylated at different sites under physiological conditions, even when considering the same kinase (e.g., glycogen synthase kinase 3). This could be due to the fact that such kinases can modify both primed (those sites that can be phosphorylated after a previous modification at a nearby site by other kinase) and unprimed sites. However, in pathological conditions, like Alzheimer's disease, a single tau isoform can be modified at an increased number of sites (hyperphosphorylated).

## B. Tau Glycosylation

Both *N*- or *O*-glycosylation of tau has been reported, with *N*-glycosylation occurring in hyperphosphorylated tau (287) whereas unmodified tau can be *O*-glycosylated (13). This relationship between phosphorylation and *O*-GlcNAc glycosylation of tau proteins may play a role in the nuclear localization of tau. Indeed, when Lefebvre et al. (183) provoked the hyperphosphorylation of tau by inhibiting phosphatase activity, the incorporation of *O*-GlcNAc into tau decreased, as did the transport of tau into the cell nucleus. Additional studies on the effect of *N*-glycosylation on the phosphorylation of tau have also been carried out (192–194).

## C. Tau Ubiquitinylation

Ubiquitin is a 76-amino acid protein that associates with proteins to be degraded in an ATP-dependent manner (129). Tau can be ubiquitinated (215), although ubiquitinated tau has mainly been found in aberrant aggregates such as inclusion bodies found in Pick's or Parkinson's diseases (206) or in some types of paired helical filaments (PHF) found in AD (216).

## D. Tau Glycation

Proteins with slow turnover rates can be modified at lysine residues by nonenzymatic reactions involving the condensation of a sugar aldehyde or ketone group with the  $\epsilon$ -NH<sub>2</sub>-groups of the lysines (201). The products of this reaction can undergo irreversible changes to form the advanced glycation end products that can result in the cross-linking of the modified proteins (71, 176). Tau isolated from PHF is glycated (175, 303), and this glycation might be involved in the aggregation of PHF into more complex aggregates (neurofibrillary tangles) (174). Moreover, it has been found that the introduction of glycated tau into cultured cells could generate oxygen free radicals capable of disturbing neuronal function (258).

## E. Tau Truncation and Deamidation

Tau truncation has been defined as the cleavage of tau that occurs at the glutamic acid residue 391 (297). This modification could facilitate aberrant tau aggregation (297). The deamidation of tau at asparagine (or glutamine) residues has also been described (289) and could also play a role in tau aggregation (213).

## F. Tau Oxidation

The presence of one or two cysteines in the tau isoforms lacking or containing exon 10 has raised the

possibility of tau forming dimers through the formation of intermolecular S-S bonds. In this case, the oxidation of tau could result in its aberrant aggregation (250). However, in the presence of exon 10, the possibility also exists of intramolecular S-S bonds forming. Recently, and in relation to tau oxidation, tyrosine nitration has been described in a tau molecule (202) or the formation of dityrosine cross-linkings (80).

## G. Other Modifications of Tau

It has been proposed that tau may become cross-linked through the enzymatic reaction modulated by transglutaminase (69, 207, 224). Moreover, modifications involving a conformational change of tau protein, and that could involve proline *cis-trans* isomerization, could also occur (290).

## VII. TAU TURNOVER

A major factor that determines the half-life of a protein is the presence of signals that control its degradation and stabilization. Among the signals for degradation, the presence of a specific amino-terminal residue, the PEST sequence, and the destruction box must be considered (54). Of the stabilization signals, amino acid repeats containing polyglutamine, glycine, or alanine residues are among the most common (54). PEST sequences are present in the tau molecule, while the tubulin-binding region is a glycine-rich sequence. However, little is known about the implication of these two regions in the stability of the tau protein.

The degradation of tau by different proteases has been studied *in vitro*, for example, using the lysosomal enzyme calpain (161). Phosphorylated tau is more resistant to proteolysis by calpain degradation than unphosphorylated tau (286). In the same way, monoubiquitinated tau (or with the addition of fewer than 5 moieties) can be subjected to lysosomal degradation (219). It is unclear whether polyubiquitinated tau could be degraded by the ATP-dependent proteasome pathway (26S proteasome); nevertheless, there is evidence that tau can be degraded by the 20S proteasome without ubiquitylation (55). While proteins like  $\beta$ -catenin need to be phosphorylated by GSK3 to be degraded, it is not known if this is also the case for the tau protein. Finally, tau cleavage by caspases has also been reported recently (243).

## VIII. TAU-ASSOCIATED PROTEINS

Because of the number of proteins associated with tau, it can be considered as a sticky protein. Of the proteins that associate with tau, tubulin is the best known

and most widely studied. Indeed, it is now almost 20 years since the binding of tau to a site in the acidic carboxy-terminal region of tubulin was demonstrated (253). Through this acidic site, tubulin binds to the basic binding region present in the tau molecule, probably through an ionic interaction (e.g., to the double lysine motif at residues 280 and 281; Ref. 102). Besides tubulin, tau can also bind to other proteins (19) such as spectrin (36), actin (50, 110), PP1 and PP2A (189, 260), kinases involved in its posttranslational modification like CDK5 (259), presenilin 1 (PS1; through a region where PS1 also binds to the kinase GSK3; Ref. 267),  $\alpha$ -synuclein (158), phospholipase C- $\gamma$  (142, 157, 180), the regulatory subunit p85 $\alpha$  from phosphatidylinositol 3-kinase (240), the fyn tyrosine kinase (168, 178), mouse Eed protein C (81), and a family of proteins expressing the Alu sequence (136). Another tau binding protein is ferritin (16), which binds to aggregated tau in a metal-dependent manner (R. Cuadros, M. Perez, and J. Avila, unpublished results).

Some of these interactions may take place at the amino-terminal region, where the PPXXP motifs that can bind to SH3 domains exist, and might be responsible for these interactions. Alternatively, these interactions may arise within the tubulin-binding region, producing obvious competition between tubulin and other tau-associated proteins.

### A. Binding of Tau to Chaperones

The phosphorylation of tau could promote its binding to the chaperone protein Pin-1, which might facilitate its posterior dephosphorylation by PP2A (198). Similarly, tau may bind to the protein 14-3.3, which binds to phosphoproteins. However, it appears that tau may bind to protein 14-3.3 in its unphosphorylated form (123), probably due to the presence of an acid region at the carboxy-terminal end of the 14-3.3 protein (277). The heat shock proteins HSP70 and HSP90 can also bind to tau, and as a consequence of this interaction, the association of tau protein with microtubules increases, decreasing its self-association and hence the formation of tangles (63).

## IX. TAU FUNCTION

As a forethought to considering the function of the tau protein, we must bear in mind that many of the studies to determine tau function have been performed through the overexpression of this protein, for example, after cDNA transfection. Thus it is possible that some of the results obtained may over exaggerate the role of tau in some processes. Nevertheless, from the pioneering work of Weingarten et al. (291), it was clear that tau facilitates tubulin assembly. Subsequently, and also through in vitro analyses, it was found that tau both stabilizes polymerized

microtubules and nucleates microtubules (27, 64, 66, 200) and that tau could suppress microtubule dynamics (27, 227).

The role of tau in stabilizing microtubules was further emphasized when, by depleting tau using antisense oligonucleotides, it was seen that tau is involved in neurite outgrowth (34, 35). Additionally, in nonneuronal Sf9 cells, the expression of tau induced the formation of long cytoplasmic extensions (169), and in other nonneural cells tau expression resulted in microtubule stabilization and bundling (170, 179). In this regard, it is noteworthy that tau can confer microtubule stability against microtubule poisons (20). On the other hand, since the tau binding site on the tubulin molecule overlaps with that for other proteins like the molecular motor kinesin, tau could also influence processes like axonal transport (72, 274).

A tau-deficient mouse, produced by gene targeting, has been seen to be viable (118), and the differences that have been identified with respect to the wild-type are restricted to a decrease in the number of microtubules in small caliber axons (118), muscle weakness, and some behavioral deficits (145). This may be explained by the compensation for the lack of tau by other proteins, and indeed, an increase of the expression of cerebellar MAP1A has been found in mice lacking tau (118). Furthermore, in cultured cells redundancy was observed between tau and MAP1B with regard to axonal growth (61, 101). In support of this hypothesis, defects in axonal elongation were found in mice lacking both MAP1B and tau (268).

Little is known about the possible role of tau as a membrane-associated protein (16, 26) or whether the nuclear protein related to tau could play a role inside the nucleus (25, 109, 195), such as in the regulation of gene expression or through its association with proteins like Edd (81). As indicated, tau also binds to many different proteins with diverse functions, and it may therefore play a role in the wide variety of processes in which those proteins are involved.

## X. TAU PATHOLOGY

Alterations in the amount or the structure of tau protein can affect its role as a stabilizer of microtubules, as well as some of the processes in which it is implicated. Changes in microtubule organization can affect the localization and organization of other subcellular structures like mitochondria (221, 269) or lysosomes (47), alterations that could provoke pathological effects. The modification of tau by phosphorylation affects its interaction with microtubules, and indeed, hyperphosphorylated tau is the essential component of the different aberrant aggregates found in neurons (and sometimes in glia) of patients with neurological disorders considered as tauopathies.



The best-known tauopathy is AD, in which two main pathological structures form in the brains of patients: senile plaques (composed of the  $\beta$ -amyloid peptide), and neurofibrillary tangles (NFT). These NFT are made up of paired PHF comprised of hyperphosphorylated tau (112, 144). The number of NFT has been correlated with the degree of dementia in this disease (17), and thus there is great interest to know how these structures form. The formation of PHF from tau molecules may follow different steps and could involve tau phosphorylation (although this may not be essential), a conformational change in the protein, and finally polymerization. If indeed phosphorylation does facilitate tau assembly into PHF, it will be of interest to know which kinases (and phosphatases) permit such levels of phosphorylation of tau molecules to be reached.

Among the kinases known to phosphorylate tau, a major role has been assigned to GSK3. In a transgenic *Drosophila melanogaster* model, it was found that GSK3 phosphorylation of tau facilitates its aggregation into filamentous polymers (156). The  $\beta$ -amyloid peptide ( $A\beta$ ) is also involved in augmenting the phosphorylation of tau by GSK3 (304), probably by increasing the enzymatic activity of the kinase (7), which only proves to be toxic for neurons in which tau protein is present (239). This increase in tau phosphorylation by GSK3 could be due to the antagonistic action of  $A\beta$  on the insulin receptor that promotes GSK3 activation (300). Additionally, Hoshi et al. (139) identified spherical aggregates of  $A\beta$  that are highly neurotoxic and that activate GSK3, which then phosphorylates tau. Other factors such as fibroblast growth factor (FGF) might also regulate GSK3 activity (124, 272), and apolipoprotein E (ApoE) and reelin may also modulate tau phosphorylation through an ApoE receptor/disabled1 (Dab1)/GSK3 cascade (226). Indeed, it has been shown that the absence of Dab1 could facilitate tau hyperphosphorylation (28), suggesting a relationship between ApoE receptors and tau phosphorylation.

Interestingly, the phosphorylation of tau by GSK3 could be needed for the formation of tau polymers (232, 233). Indeed, in an attempt to mimic the hyperphosphorylation of tau seen in AD, which is at least in part due to GSK3, neural cells have been treated with phosphatase 2A (and phosphatase 1) inhibitors like okadaic acid (232). If the cells are additionally treated with hydroxynonenal (HNE), tau filaments form. Recently, it has been found that the *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine reverses okadaic acid-induced hyperphosphorylation, presumably by acting on PP2A (188). However, it is possible that the phosphorylation of tau by GSK3 at specific sites could require prior phosphorylation by other kinases at adjacent sites to those to be modified by GSK3 (primed phosphorylation; Ref. 257). Thus other tau kinases have been studied (256, 257), as has the regulation of tau phosphatases (99). With respect to the

regulation of tau phosphatases, it should be born in mind that PP2A function and assembly is dependent on its carboxy-methylation (279).

The phosphorylation of tau may promote a conformational change that can be functionally reversed in the presence of trimethylamine *N*-oxide (TMAO), a natural occurring osmolyte (278). This conformational change can also be reversed by the chaperon protein Pin-1 (198), a molecule that upon tau binding facilitates the posterior action of PP2A in dephosphorylating the protein (308). These conformational changes could facilitate tau aggregation (Fig. 4), and recently, it has been suggested that such conformational changes (or others) may result in an increase of  $\alpha$ -helices in the secondary structure of tau, since the content of  $\alpha$ -helices is greater in tau from PHF (107, 159, 208, 248). However, it has also been suggested that filament formation is also partially dependent on the presence of certain  $\beta$ -sheet structures within tau (284). It would be of interest to know whether the tau present in other aggregates such as Pick's bodies adopts a different conformation. Nevertheless, it does seem that a conformational change is undertaken when tau polymerizes into PHF (1, 78). Furthermore, it appears that this conformational change could involve the binding of the amino-terminal region of the tau molecule to its microtubule binding region (37).

Two of the protein kinases that can modify tau were found to bind to microtubules, tau protein kinase I and tau protein kinase II (146, 153). These kinases correspond to GSK3 and cdk5, respectively (152). The importance of these kinases in AD has been discussed above, and it has been shown that prior phosphorylation of tau by kinases like tau kinase II enhances its subsequent phosphorylation by tau kinase I (151), emphasizing the importance of tau kinase II (cdk5) on tau phosphorylation in AD (122). Indeed, it has been proposed that calpain activity and the levels of p25 (an activator of cdk5 produced from the p35 precursor protein by calpain proteolysis) are increased in AD (230), although this is not altogether clear (271, 306).

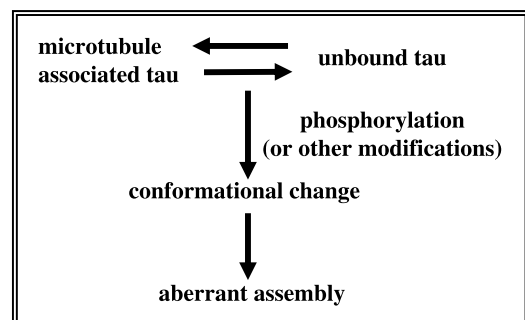


FIG. 4. A model for paired helical filaments (PHF) and neurofibrillary tangles (NFT) formation. The presence of tau that is not bound to microtubules may result in its phosphorylation and, subsequently, in its assembly into polymers like PHF. These polymers could aggregate to form NFT.

In transgenic mice overexpressing p25, tau hyperphosphorylation does occur (230). However, when calpain is activated in neural cells by glutamate treatment, tau phosphorylation decreases (164), probably due to the simultaneous activation of calcineurin (a calcium-dependent phosphatase).

## XI. TAU ASSEMBLY IN VITRO

As early as 1986, Montejo et al. (212) described that purified tau protein can form fibrillar polymers resembling the PHF found in the brain of AD patients and that a modification such as deamidation could facilitate this polymerization (210–212). Curiously, several years later it was shown that deamidation occurs in tau obtained from PHF (288). In vitro, tau assembly has been further studied (53, 276, 293, 295), and it has been shown that a high concentration of protein is needed for tau to polymerize (53), suggesting that other compounds could be necessary to facilitate tau assembly. The sulfoglycosaminoglycans (sGAGs), which are present along with tau in NFT, were some of the first molecules tested. It was found that sGAGs facilitate tau polymerization in vitro independently of the phosphorylation state of tau (88, 235). sGAGs are polyanions, and other polyanions such as the glutamic acid-rich region present at the carboxy-terminal region of tubulin can also facilitate aggregation, this aggregation requiring the presence of the third tubulin binding motif of the tau molecule (235).

It has also been suggested that oxidation could play a role in tau aggregation. Oxidation of cysteine to produce disulfide cross-linking favors tau self-assembly in tau 3R molecules, where a single cysteine is present, but not in tau 4R molecules, where the presence of two cysteines may permit the formation of intramolecular disulfide bonds (22). In vitro studies have shown that tau can be assembled through oxidation processes (Fenton's reaction) in the presence of iron (276). Also, fatty acids like arachidonic acid can induce tau polymerization in vitro (1, 295). This type of polymerization could be related to the possible interaction of tau with plasma membrane components (15, 26).

Anionic micelles and vesicles induce tau fibrillation in vitro (42). In addition, it has been suggested that tau could play a role in the activation of phospholipase C (PLC)- $\gamma$  to generate arachidonic acid through the hydrolysis of phosphoacetylcholine (142). In fact, an interaction between PLC- $\gamma$  and tau protein has already been described (157). The arachidonic acid generated could facilitate tau aggregation (1), possibly since the arachidonic acid micelles can act as polyanions since their negative charged carboxyl groups are exposed at the surface. It is noteworthy that lipid peroxidation occurs in AD, and a compound like arachidonic acid could be fragmented to

yield toxic products like HNE. Recently, it has been shown that HNE facilitates tau assembly, but only if tau is hyperphosphorylated (232, 233), and that such a modification is in part due to GSK3 phosphorylation. On the other hand, it has been proposed that carnosine can quench the effect of HNE (3), and in this context, antioxidants like *N*-5 butyl hydroxylamine could be used as neuroprotectors (18).

Finally, some quinones could also induce tau polymerization in fibrillar form (I. Santamaría, F. Moreno, and J. Avila, unpublished data), and  $\alpha$ -synuclein has also been seen to facilitate tau assembly (79).

### A. Phosphorylation Before or After Assembly

A further question that arises from the possible link between tau phosphorylation and assembly is whether tau phosphorylation occurs before or after PHF assembly? If tau phosphorylation occurs first, and it occurs in a region that will be masked after PHF assembly, the site will not be accessible to phosphatases following assembly, and we can hypothesize that such a site was phosphorylated before tau assembly. The antibody (Ab) 12E8 recognizes a phosphoserine (S262) located in one of the tubulin binding motifs present in tau molecule. The binding of this Ab to tau is augmented in AD extracts; however, this Ab does not react with assembled PHF (127). This suggests that tau phosphorylation occurs before its assembly, a hypothesis that is supported by other studies (104). Indeed, strong evidence exists that the phosphorylation of tau promotes its self-assembly (5).

### B. Toxic Effects of Modified Tau

The cytotoxicity mediated by tau in AD could be due to its hyperphosphorylation or to the formation of aberrant aggregates. In the first case it has been observed that expression of pseudohyperphosphorylated tau promotes toxicity associated with the induction of apoptotic cell death (73). This result is in agreement with observations in GSK3 transgenic mice (126, 199).

In AD, there is an inverse correlation between the number of extracellular tangles and the number of surviving neurons, suggesting that neurons developing neurofibrillary lesions could degenerate (82). On the other hand, the appearance of extracellular NFT occurs in those regions that contain neurons with intracellular NFT. This suggests that intracellular inclusions precede cell death and that extracellular NFT form as a result of cell lysis, perhaps due to the binding of tau to extracellular matrix components like sGAGs (235). Thus the presence of tau aggregates could be postulated as toxic and could result from the fact that tau aggregates are sticky structures that could bind to, and therefore deprive the cell of, proteins

needed for normal metabolism like ferritin (128) or Pin-1 (198). It has also been proposed that abnormally hyperphosphorylated cytosolic tau might sequester other MAPs like MAP1B or MAP2. Such sequestering may result in the destabilization and disassembly of microtubules, and in the appearance of morphological changes that could promote the disruption of synaptic contacts (6, 149).

### C. PHF Morphology

The morphology of the PHF, mainly the proportion of helices, has been extensively studied. Thus it has been proposed that PHF morphology may depend on the proportion of tau isoforms containing three or four tubulin binding motifs (88), on the association with sGAGs (16), or on the presence of specific residues present in tau molecule (58). Since the pioneer paper of Kidd (167), different techniques have been applied to characterize the structure of PHF, including X-ray diffraction (298), electron microscopy, high-resolution transmission electron microscopy (TEM), atomic force microscopy, or cryoelectron microscopy (12, 211, 214, 236, 244, 245, 296). The results suggest that the structure of PHF is compatible with a helical ribbon made up of two parallel strands. The formation of NFT from PHF appears to be facilitated by glycation (174; Fig. 4).

## XII. TAUOPATHIES

Tauopathies are considered as a group of disorders that are the consequence of abnormal tau phosphorylation, abnormal levels of tau, abnormal tau splicing, or mutations in the tau gene. In some tauopathies, like AD or Down's syndrome, the tau pathology is associated with other cerebral changes.

### A. AD

AD is the most common and the best-studied tauopathy. The disease results in widespread atrophy in the brain that begins in the temporal and parietal lobes. It generates problems in recent memory, a function associated to the temporal lobe, and in visual and spatial dysfunction and poor performance of over-learned tasks, functions of the parietal lobe (292). Tau aggregates from AD are composed of the six CNS tau isoforms in their phosphorylated form (see Fig. 2). The six tau isoforms are as follows: 1) containing exons 2, 3, and 10, plus all the constitutive exons (see sect. II; Ref. 21); 2) having exons 2 and 3; 3) containing exons 2 and 10; 4) having only exon 2; 5) with only exon 10; and 6) only containing the constitutive exons. This combination of hyperphosphorylated tau isoforms results in the appearance of three major

electrophoretic bands with a mobility corresponding to that of proteins with a relative molecular weight of 68,000, 64,000, and 60,000 (56, 91, 108, 181). Furthermore, AD brains contain higher quantities of tau than unaffected controls (165, 166).

Below is a summary of other well-studied tauopathies [for more specific details see the excellent reviews by Buee et al. (32) and Ingram and Spillantini (147)].

### B. Corticobasal Degeneration

Corticobasal degeneration is a disorder characterized by cognitive disturbances like aphasia and apraxia, moderate dementia, and motor disturbances such as rigidity, limb dystonia, and tremor. Pathological analyses have indicated frontoparietal atrophy and glial and neuronal tau inclusions. Tau is also present in hyperphosphorylated form, but only the 68,000- and 64,000-molecular weight forms can be detected by electrophoresis.

### C. Down's Syndrome

Down's syndrome is due to the trisomy of chromosome 21 that results in the defective growth and maturation of the brain, producing a cognitive impairment and dementia at ~50 years of age. In this disorder, tau is also hyperphosphorylated yielding a pattern similar to that of AD.

### D. Frontotemporal Dementia With Parkinsonism Linked to Chromosome 17

In this disorder, the patient displays frontotemporal atrophy, with neural loss, gliosis, and cortical spongiform changes in the lobes. This results in behavioral changes, language deficit, and hyperorality. Tau inclusions were observed in neuron and glia cells, and two types of hyperphosphorylated tau forms were detected. In some cases the pattern is similar to that of AD, the 68,000-, 64,000-, and 60,000-molecular weight forms being detected, while in other cases the pattern is like CBD and only the 68,000- and 64,000-molecular weight forms were found. This is due to intronic mutations that result in the forced expression of exon 10 (111, 141, 261, 282). On the other hand, mutations resulting in the deletion of lysine-280 lead to reduced splicing of exon 10 (242), while the G342V mutation may affect the splicing of exons 2 and 3 (191). Some factors involved in the alternative splicing of tau have been already identified (68), and more than 25 mutations in tau have been identified in exons 1, 9, 10, 11, 12, and 13. These mutations may effect RNA splicing or the protein levels of tau. These tau (missense) mutations preferentially occur in the microtubule binding region

(exon 10), decreasing binding of the mutated protein to microtubules (121, 138) or affecting the binding of tau to other proteins that bind to that region of tau (90). Furthermore, tau self-assembly was also increased in most of these mutated tau proteins (14).

### E. Pick's Disease

Pick's disease is a dementia that produces disturbances in language and behavior and is associated with frontal lobe atrophy. It provokes changes in the character of the patient and in their relationships with others, as well as depression. This disorder is characterized by the presence of cytoplasmic tau inclusions in neurons of the frontal lobe, known as Pick bodies. The granular cells of the dentate gyrus are also affected. The appearance of inclusions is in agreement with the appearance of 64,000- and 60,000-molecular weight hyperphosphorylated tau, indicating the absence of exon 10 expression. This exon is not expressed in tau present in granular cells of the dentate gyrus (94), suggesting that the degeneration of selective neuronal populations in different tauopathies reflects the physiological pattern of tau isoforms expressed.

### F. Postencephalic Parkinsonism

This disorder appears to be the consequence of an encephalic parkinsonism found in patients that survived the Spanish influenza pandemic (77). Different brain regions are affected, but tau inclusions are mainly found in the hippocampus and the putamen. The electrophoretic pattern for hyperphosphorylated tau is similar to that of AD.

### G. Progressive Supranuclear Palsy

Progressive supranuclear palsy (PSP), also known as the Steele, Richardson, and Olszewski disorder, is characterized by supranuclear gaze palsy as well as by prominent postural instability, and in the later stages by dementia. Tau inclusions have been found in neuronal and glial cells, with both astrocytes (tufted astrocytes) and oligodendrocytes (coiled bodies) being affected. Recently, as in FTDP-17, missense mutations have been found in PSP patients with a monogenic (familial) origin (57), and in fact to date, mutations in Tau have been identified in PSP (J. G. De Yebenes, personal communication), CBD (33, 237), and Pick's disease (220, 241). Some specific tau polymorphisms may be a risk factor for PSP (59, 137), and these polymorphisms are based in the different number of repeats of a dinucleotide repeat (TG) present in the intron between exons 9 and 10 (see above).

Individuals containing 11 dinucleotide repeats (tau allele A0) have been seen to have a greater risk of developing PSP.

### H. Niemann-Pick Type C Disease

Niemann-Pick type C disease is an autosomal recessive lysosomal lipid storage disorder, mainly caused by mutations within the *NPC-1* gene (280). The clinical features are motor disturbances (due to cerebellar dysfunction) and dementia (281). The pattern of hyperphosphorylated tau is similar to that of AD.

### I. Other Tauopathies

Other tauopathies involving hyperphosphorylated tau include parkinsonism with dementia, myotonic dystrophy, prion diseases with tangles, Blint disease (an ophthalmologic disorder), dementia pugilistica, dementia with tangles only or amyotrophic lateral sclerosis, and parkinsonism-dementia complex of Guam. These have been well-documented in the reviews of Buee et al. (32) and in that of Ingram and Spillantini (147).

### J. Lack of Pathology in Some Neurons

In AD, cerebellar neurons are resistant to degeneration (143, 218) and aggregates in these neurons (e.g., Purkinje cells) are not found. This may be due to the low content of tau in these neurons (31), although in diseases like Niemann-Pick type C disorder, cerebellar tau might be hyperphosphorylated (31). In PNS neurons, no aberrant tau aggregates have been found. This may reflect the presence of the extra exon 4A in the tau molecule that prevents tau self-assembly (231).

## XIII. ANIMAL MODELS

### A. Lower Eukaryotes: *Drosophila* to Lamprey

The generation of models of tauopathies in higher organisms is difficult because their genomes are not easy to manipulate. Thus lower eukaryotes, such as the fruit fly and Lamprey, have been used to analyze the mechanisms by which tau is able to contribute to neurodegeneration. A tau homolog exists in *Drosophila* (125, 285) that has 46% identity and 66% similarity with the human tau protein (125). Transgenic flies expressing human wild-type and mutant tau (R406W) both die prematurely (299), although the toxicity of the mutant tau was higher than the wild-type. Interestingly, neurodegeneration occurred without filament formation. A similar approach, overexpressing



human wild-type tau and shaggy/zeste white-3, the homolog of human GSK-3 $\beta$  in transgenic flies has also been followed (156). In these flies, neurodegeneration was exacerbated and abnormal filaments were observed showing the importance of GSK-3 in this process.

The lamprey, a lower vertebrate, has been used to study the metabolism of human tau in specific neurons by microinjecting plasmids encoding human tau. In this model, it has been demonstrated that the carboxy-terminal domain of human tau is necessary for its transport to the axon or dendrites (115). In addition, expression of human tau in anterior bulbar cells induced the formation of filaments and neurodegeneration (115). In this system, filamentous tau (straight filaments) was associated with the loss of dendritic microtubules and synapses, as well as with the disruption and aggregation of membranous organelles (113). Interestingly, these effects are independent of the tau isoform expressed (114).

## B. Vertebrates

Several approaches have been adopted in an attempt to model tauopathies in vertebrates. Thus transgenic animals for genomic human tau, as well as mice carrying cDNAs encoding either the largest or the smallest CNS isoform of human tau have been generated. In addition, transgenic mice carrying tau cDNA with the missense mutations found in FTDP-17 patients have also been obtained. Finally, an additional strategy to generate animal models of tauopathies has been to overexpress the kinases responsible for tau hyperphosphorylation.

Mice have been generated that overexpress the genomic sequence of human tau, containing the coding sequence, intronic regions, and the regulatory regions of the gene (70). The human tau is distributed in neurites and at synapses but is absent from cell bodies, and no neuropathological lesions were reported in mice of up to 8 mo of age.

In the majority of FTDP-17 patients, the polymers assembled from tau only contain the four-repeat isoforms (131), and in several FTDP-17 families, the only tau mutations found have been those that affect the splicing of exon 10, increasing the ratio of four-repeat with respect to three-repeat isoforms (131). Taking these observations into account, it was suggested that the four-repeat forms of tau may favor fiber formation, and thus mice have been generated to test this hypothesis that carry cDNAs encoding either the largest or the smallest CNS isoform of human tau (105). On the other hand, several transgenic animals overexpressing the shortest isoform have been obtained but using different transgene promoters. With the murine 3-hydroxy-methyl-glutaryl CoA reductase promoter (29), hyperphosphorylated somatodendritic transgenic tau was detected although NFTs did not appear to

form in these animals. The level of expression of the same tau isoform was increased by using the murine PrP promoter (154). Transgenic lines with high levels of overexpression were not viable while lines with less than 10-fold overexpression of tau protein developed inclusions in cortical and brain stem neurons. These inclusions were most abundant in spinal cord neurons and were correlated with axon degeneration, diminished microtubules, reduced axonal transport in ventral roots, spinal cord gliosis, and motor weakness. NFT-like inclusions (detected by histochemistry using dyes such as Congo red and Thioflavin S) were detected in the same transgenic mice at 18–20 mo of age (155). In addition, filaments were isolated from detergent-insoluble tau fractions (155).

The first transgenic mice to be published that carried cDNAs encoding the largest human brain tau isoform showed low levels (10%) of overexpression (105). Transgenic human tau protein was present in nerve cell bodies, axons, and dendrites. Tau was phosphorylated at sites that are hyperphosphorylated in PHFs, although filaments were not detected. The murine Thy 1.2 promoter has been used by two groups to express the cDNA encoding the longest human brain tau isoform, although on different genetic backgrounds. In the first case, axonal degeneration in the brain and spinal cord was detected in the mice (263). Axonal dilation with accumulation of neurofilaments, mitochondria, and vesicles was observed, and the axonopathy and accompanying dysfunction of the animals sensorimotor capacities were transgene-dosage related. The mice generated by the second group (238) contained numerous abnormal, tau-immunoreactive nerve cell bodies and dendrites. In addition, large numbers of pathologically enlarged axons containing neurofilament and tau-immunoreactive spheroids were also present, especially in the spinal cord.

The third approach to generate animal models of tauopathies is based on the mutations in tau associated with FTDP-17 (44, 85, 111). Recently two groups have reported the generation of transgenic mice expressing mutant human tau containing the P301L mutation (106, 185) that is located in the tubulin-binding domain, and that reduces the affinity of tau for microtubules. The first of the mice generated developed a spinal cord pathology and motor dysfunction. These animals developed NFTs mainly in the spinal cord, and like the previous transgenic models developed some neuropathological symptoms encouragingly reminiscent of the human disease. In the second transgenic model published, short filaments of tau could be isolated from the brains of the transgenic mice (106).

More recently, another transgenic mouse containing the P301L mutation has been characterized (249), as well as one containing the P301S mutation (4). In the latter, abundant tau filaments within a PHF structures were observed. Curiously, in the transgenic mouse expressing

the tau P301L mutation, tau filaments were only observed in old female mice but not in their male counterparts. This could be due to a decrease in the amount of tau in male mice (31). Filaments were also found in transgenic mice expressing the mutation R406W in human tau (273), a mutation that decreases the phosphorylation of tau at the site recognized by Ab PHF-1 (234). Finally, it has been shown that filaments form in transgenic mice expressing mutant (V337M) human tau (270).

In summary, a significant body of data demonstrates that a large excess of normal or mutated human tau can promote some of the cellular changes observed in tauopathies, but that this may also be insufficient for the formation of the mature neurofibrillary aggregates observed in the human diseases. Nevertheless, some transgenic lines do develop NFT-like structures as well as short PHF-like filaments. What these approaches seem to indicate is that, first, tau is linked to neurodegeneration, and second, that neurons with long axons, such as those present in spinal cord, seem to be especially susceptible to alterations in tau. We have recently produced a transgenic mouse expressing three FTDP-17 missense mutations in tau: G272V, P301L, and R406W (190). Ultrastructural analysis of mutant tau-positive neurons revealed a pretangle appearance; filaments of tau were found as well as increased numbers of lysosomes displaying aberrant morphology similar to those found in AD (190).

An additional strategy for generating animal models of tauopathies has been to overexpress the kinases responsible for tau hyperphosphorylation. The first lines of GSK-3 $\beta$  transgenic animals described used either ubiquitous (murine sarcoma virus) or CNS-specific promoters (murine neurofilament light chain) to drive expression (30). Although slight increases in tau phosphorylation at the AT8 epitope were detected, overexpression was not observed using transgenes encoding either wild-type GSK-3 $\beta$  or a mutant GSK-3 $\beta$  (S9A) that is not inhibited by phosphorylation. The authors speculated that the low levels of kinase expression obtained were probably because high levels of GSK-3 are lethal. Conversely, GSK-3 $\beta$  knock-out mice die during embryonic life (135).

The second transgenic animal published also expressed a constitutively active form of the kinase, i.e., GSK-3 $\beta$ (S9A), under the control of the *thyl* gene promoter (262). An increase in phosphorylated tau was demonstrated but only in older transgenic mice (6–7 mo). These GSK-3 $\beta$  transgenic mice performed normally in the Morris water maze test, but interestingly, when cross-bred with transgenic mice that overexpress the longest human protein tau isoform, the number of axonal enlargements present and the motor impairment typical of these tau transgenic animals were reduced (264). In these mice, an increase in the phosphorylation of human tau was observed, although neither an increase in insoluble tau ag-

gregates nor the presence of paired helical filaments was observed.

With the lethality of GSK-3 $\beta$  (30) as well as the known role of GSK-3 $\beta$  in development taken into account, a further GSK-3 $\beta$  transgenic mouse was generated (199) using the conditional tetracycline-regulated system under the control of the CaM kinase II  $\alpha$ -promoter to achieve substantial overexpression of the kinase. Animal models of neurodegeneration using the tetracycline-regulated system have the advantage of being able to perform reversible studies (301). In this transgenic mouse (tet/GSK-3 $\beta$ ), GSK-3 $\beta$  overexpression was restricted to certain cortical neurons and hippocampal neurons of the dentate gyrus and CA2 regions. In vivo overexpression of GSK-3 $\beta$  resulted in an increase in the phosphorylation of tau in tet/GSK-3 $\beta$  animals, as detected with antibodies raised against the phosphorylated tau modified in AD. Hyperphosphorylated tau was found in the somatodendritic compartment, similar to the localization of tau previously observed in tau transgenic mice. However, the change in the subcellular localization of tau observed was exclusively due to the hyperphosphorylation of tau by GSK-3 $\beta$  since there was no change in the overall levels of tau. Somatodendritic hyperphosphorylated tau in the conditional tet/GSK-3 $\beta$  transgenic mice produced an increase in the levels of tau not bound to microtubules in this neuronal compartment, immunoreactivity to 7.51, an antibody that recognizes the tubulin-binding domain of tau increased. These data suggest that the increased phosphorylation of tau results in a decrease in the affinity of tau for microtubules, reproducing two of the characteristics of AD tau: hyperphosphorylation and decreased interaction with microtubules. However, the aberrant tau aggregations found in AD were not observed in these GSK-3 $\beta$  transgenic mice.

In tet/GSK-3 $\beta$  mice,  $\beta$ -catenin, another GSK-3 substrate relevant to AD (184), was also analyzed. GSK-3 is a key enzyme involved in  $\beta$ -catenin stabilization and nuclear translocation (8). In the Tet/GSK-3 $\beta$  mice, GSK-3 $\beta$  was seen to be effective in modifying  $\beta$ -catenin in central neurons in vivo, since in hippocampal granular cells of tet/GSK-3 $\beta$  mice nuclear  $\beta$ -catenin was reduced by ~75% (199). Considering that the genes transactivated by  $\beta$ -catenin are poorly characterized, tet/GSK-3 $\beta$  mice may serve as a good tool to identify such genes. Tet/GSK-3 $\beta$  mice also demonstrate neuronal stress and neuronal death as revealed the presence of reactive glia, TUNEL labeling, and cleaved caspase-3 staining. These data are in agreement with the known role of GSK-3 $\beta$  in the survival pathway, as well as supporting the neuroprotective effect of lithium, a relatively specific GSK-3 $\beta$  inhibitor (39). Thus these in vivo genetic approaches support the involvement of GSK-3 $\beta$  in tau phosphorylation in the nervous system, suggesting that GSK-3 deregulation might be relevant to the pathogenesis of AD.

Hyperphosphorylation of hippocampal tau in transgenic Tet/GSK3 mice, despite the lack of filament formation, results in a behavioral impairment that can be measured in the Morris water maze test (126). Thus hyperphosphorylation of tau could be sufficient to provoke some of the pathological manifestations found in AD. At present, it is not clear if these aspects could be reversed or not. If they can be reversed, this might suggest that tau aggregation may be the cause of a nonreversible toxicity such as that present in AD neurons. Furthermore, GSK-3 appears to be involved in neurodegeneration (see above), and as such, crossing tet/GSK-3 $\beta$  with tau transgenic mice should improve the chances of obtaining PHFs. These tet/GSK-3 $\beta$  mice could also prove to be a good tool to test the neuroprotective effect of forthcoming GSK-3 $\beta$  specific inhibitors. The efficacy of such inhibitors could be compared with the effect of silencing transgene expression by administering tetracycline analogs in a mouse conditional model (199).

In summary, the two transgenic mouse lines that overexpress GSK-3 $\beta$  in the brain show an increase in tau phosphorylation, although that generated by Spittaels et al. (264) shows neither an overt phenotype nor evidence of neuronal degeneration. However, the model generated by Lucas et al. (199) shows spatial learning deficits and hippocampal neurodegeneration. Among the many possible explanations for this discrepancy are constitutive versus conditional expression, the promoter used, wild-type versus constitutively active form of GSK-3 $\beta$ , genetic background, etc. However, it still remains a possibility that tau may be irrelevant to neuronal death induced by increased GSK-3 $\beta$  and to neurodegeneration in general and that GSK-3 $\beta$  overexpression can either promote or prevent neuronal degeneration (204). Nevertheless, while it remains unproven that neuronal death is a consequence of hyperphosphorylation of tau in either Tet/GSK-3 $\beta$  mice or in AD, the fact that tau mutations are responsible for FTDP-17 clearly establishes that tau modifications are sufficient to cause neurodegeneration.

The idea that "overexpression of GSK-3 $\beta$  can either promote or prevent neuronal degeneration" (199) derives from the fact that the transgenic mouse overexpressing GSK-3 $\beta$  was not initially analyzed as such, but rather in the context of the overexpression of tau (264). Thus considerable expression of the tau protein results in transgenic mice that develop an axonopathy, while transgenic mice with a moderate tau overexpression do not exhibit neurodegeneration (262, 264). The most likely explanation for this is that an excess of tau bound to microtubules inhibits axonal transport (72). This is in agreement with the fact that the axonopathy in tau overexpressing mice occurs preferentially in neurons with long axons such as brain stem and spinal cord motor neurons. Because phosphorylation of tau by GSK-3 $\beta$  decreases the affinity of tau for microtubules, Spittaels et al.

(264) concluded that the most likely explanation for the improvement of this axonopathy and the motor problems found in httau40 mice was the rescue of the axonal transport that was inhibited by the excess of the MAP tau. Thus GSK-3 $\beta$  is able to attenuate the pathological consequences of the massive overexpression of one of its substrates, tau, which is not surprising. However, in the absence of such aberrant high levels of tau in vivo (199) and in vitro (130, 186, 228), the excess of GSK-3 $\beta$  activity results in neuronal death.

#### **XIV. A WORKING HYPOTHESIS TO EXPLAIN TAU PATHOLOGY DUE TO TAU HYPERPHOSPHORYLATION OR TAU FILAMENT FORMATION**

Pathologies in which tau is implicated could initially be the result of the presence of hyperphosphorylated tau. In a nonpathological situation, different isoforms of tau can be phosphorylated at different residues by an individual kinase such as GSK3 (Fig. 3). This could be due to the localized presence of kinases within the cell that phosphorylate tau at specific sites, these phosphorylation sites facilitating the further phosphorylation by GSK3 (primed phosphorylation; see Ref. 75). Furthermore, the presence of other molecules that could bind to different phosphorylated regions of the tau molecule in specific cell compartments could explain the fact that a single tau isoform is not hyperphosphorylated at multiple sites, since some of these may be masked by the binding of such molecules.

In a pathological situation there is a simultaneous phosphorylation of a single tau isoform at different sites, resulting in a dramatic change in its electrophoretic mobility. This hyperphosphorylation could be maintained if phosphatases like PP2A were not functioning properly (Fig. 5). Such a failure to activate these phosphatases may result from changes in their carboxymethylation state, possibly through a complex pathway involving homocysteine and vitamins like B<sub>12</sub> or B<sub>6</sub> (279). Other possible mechanisms for PP2A failure could involve the decreased binding of the phosphatase to the tau molecule, which may result from the mutation of tau at certain residues as described in some FTDP-17 patients (90). Also hyperphosphorylation of tau could provoke conformational changes that inhibit phosphatase function, and it is possible that chaperones like Pin-1 could partially reverse such aberrant conformations and promote phosphatase activity.

Thus hyperphosphorylated tau could be assembled into PHFs, in the presence of other compounds that might facilitate polymerization. These tau aggregates may be toxic to the neuron that in turn might lose its synaptic contacts (217). After a period of survival (217), neuronal death could occur, followed by cell lysis that would liberate tau into the extracellular space where it could bind



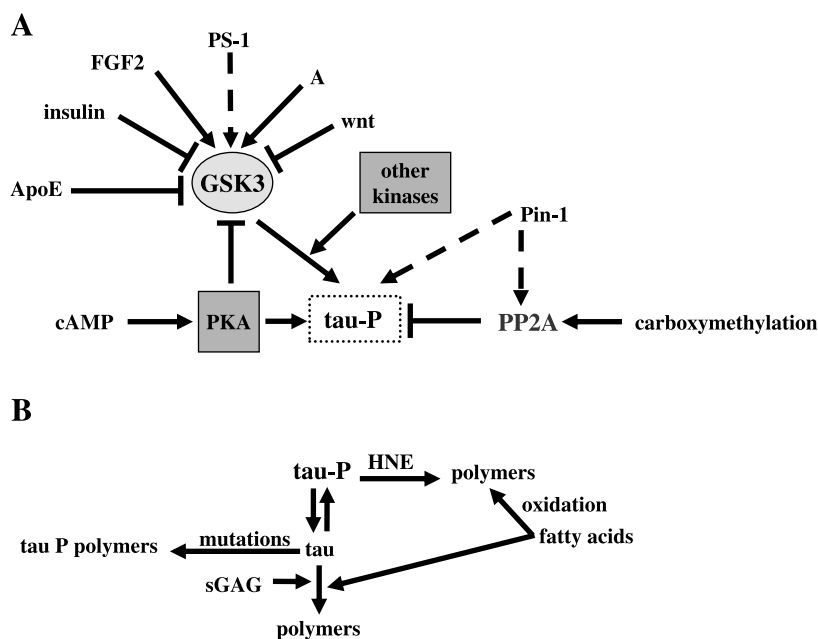


FIG. 5. *A*: tau phosphorylation and assembly. Tau may be phosphorylated by different kinases. One of these kinases, glycogen synthase kinase 3 (GSK3), may play an important role in either primed or unprimed phosphorylation events. GSK3 activity can be regulated by different extracellular factors like insulin, A $\beta$ , wnt, etc., or by phosphorylation by other kinases such as protein kinase A (PKA). On the other hand, dephosphorylation may be achieved through different phosphatases, although protein phosphatase 2A (PP2A) appears to play an important role. This phosphatase can also be regulated by a variety of cellular factors. *B*: phosphorylated tau can be assembled in the presence of compounds such as hydroxynonenal (HNE), a molecule resulting from the oxidation of fatty acids. Other factors that might facilitate tau polymerization include specific mutations found in tau from FTDP-17 patients, the presence of sulfoglycosaminoglycans (sGAG) or other polyanions, and fatty acids.

to molecules like sGAG that will promote its polymerization. These polymers, upon glycation, could assemble into extracellular NFTs.

In other tauopathies, kinases other than GSK3, like stress kinases (95, 120), could play an important role in the hyperphosphorylation of tau, also resulting in its aggregation (in the presence of peroxidation products like HNE), although in neurons localized at sites different to those damaged in AD.

## XV. SUMMARY

Tau is a microtubule-associated protein that is not essential for mammalian development, probably due to functional redundancy and the presence of other microtubule-associated proteins. However, in pathological situations tau may be hyperphosphorylated and assembled in an aberrant way. As a consequence of these modifications, neural toxicity augments, resulting in the appearance of neurological disorders, mainly dementias, which are collectively known as tauopathies. Different types of neurons are damaged in different tauopathies, and the elucidation of the mechanisms underlying the basis for this specificity will probably be the objective of several laboratories working in neurodegeneration.

We are grateful to Drs. J. Díaz-Nido, F. Wandosell, and F. Lim for helpful comments on the manuscript. We are also indebted to S. Soto-Largo for excellent assistance in the preparation of the manuscript.

Our work was supported in part by grants from Spanish Comision Interministerial de Ciencia y Tecnología (CICYT), Comunidad de Madrid, Fondo de Investigaciones Sanitarias Seg-

uridad Social (FISS), NeuroPharma, F. Ramón Areces, and Fundación Lilly.

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