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Cellular and Molecular Life Sciences

Tauopathies

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Abstract. Tau is a microtubule-associated protein predominantly expressed in nerve cells that promote microtubule assembly and microtubule stabilization. Tau is a cytosolic protein mainly present in axons and involved in anterograde axonal transport. In several neurodegenerative diseases, as for example Alzheimer's disease, tau metabolism is altered. Thus, alterations in the amount of the tau protein, missense mutations, posttranscriptional modifications like phosphorylation, aberrant tau aggregation or a different expression of some of its isoforms could provoke pathological effects resulting in the appearance of neuronal disorders known as tauopathies. The purpose of this work is to review the possible mechanisms for tau alterations that could lead to the onset of tau pathology. First we will focus on tau turnover, then on tau phosphorylation and, finally, on tau aggregation.

Keywords. Tauopathies, Alzheimer's disease, FTDP-17, tau, paired helical filaments.

Tau protein

Tau was first isolated as a microtubule-associated protein, since it copurifies with microtubules (for review, see [1]). The binding of tau to microtubules was found to be through specific microtubule-binding domains (MBDs). These domains are three or four imperfectly repeated sequences of 31 or 32 residues located in the C-terminal half of the tau molecule [2–4]. Apart from these MBDs, two proline-rich regions whose phosphorylation affect the ability of tau to bind microtubules flank MBDs. Serine and threonine residues present in both regions are modified by different protein kinases. Tau is a protein predominantly expressed in nerve cells that promote microtubule assembly and microtubule stabilization [5]. Tau is a cytosolic protein mainly present in axons, although it can be also found associated to the cell membrane [6, 7]. Tau is important in neurogenesis, axonal maintenance and axonal transport.

About 20 years ago it was found that tau was the main component of paired helical filaments (PHFs) [8–13] and that purified tau was able to assemble in vitro into fibrillar polymers that resemble the morphology of *ex vivo* PHFs [14]. Subsequent studies revealed that PHFs were rich in a phosphorylated protein [15], which turned out to be a hyperphosphorylated form of tau [16, 17]. Aberrant phosphotau polymers also appear associated with other neurological diseases like Pick's disease, frontotemporal dementia linked to chromosome 17 (FTDP-17), corticobasal degeneration, progressive supranuclear palsy, Guam parkinsonism dementia complex, dementia with argyrophilic grains, Niemann-Pick disease type C and dementia pugilistic [18]. These disorders are collectively named tauopathies (Table 1).

Table 1. Tauopathies involving hyperphosphorylated tau.

Alzheimer'disease: sporadic and familial FTDP-17
Progressive supranuclear palsy
Corticobasal degeneration
Down's syndrome
Pick's disease
Guam parkinsonism dementia complex
Dementia with argyrophilic grains
Niemann-Pick disease type C
Dementia pugilistic

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Synthesis of tau protein

Tau gene is located on human chromosome 17 where it occupies over 100 kb and contains 16 exons [19, 20]. Upstream of the first exon there is a region that contains consensus binding sites for promiscuous transcription factors such as AP2 and SP1. Since tau is mainly a neuron protein, the presence of specific neuronal factors involved in the transcription of tau gene has been proposed [21]. On the other hand, the presence of RNA molecules that could regulate tau expression was recently suggested [22]. Thus, there are transcripts from a gene, found in the tau locus, which overlaps with and regulates tau gene expression being a natural antisense gene that regulate tau gene expression.

Tau gene is transcribed into nuclear RNA, which by alternative splicing yields different RNA species that, upon translation, result in the expression of different tau isoforms with different numbers of exons. In the adult brain, six isoforms of tau are expressed [2]. The isoforms differ in the presence or absence of a fourth 31-amino acid repeat, coded by exon 10, in the MBD, as well as in the presence in the amino terminal part of tau protein of zero, one or two inserts (coded by exons 2 and 3). Thus, exons 2, 3 and 10 are alternatively spliced (Fig. 1). Exon 2 can appear alone, but exon 3 never appears independent of exon 2 [23]. In the peripheral nervous system, there is a high-molecular-weight tau isoform expressing exon 4A, which yields a protein known as big tau with an approximate size of 100 kDa [24, 25]. Because exon 10 encodes one of the regions involved in the binding of tau to microtubules, alternative splicing of exon 10 produces tau isoforms, with either three (tau 3R without exon 10) or four (tau 4R with exon 10) MBDs. The proportion of these tau isoforms, as well as their phosphorylation status, changes during development [26–31]. Thus, the smallest isoform, without the two N-terminal inserts and with only three MBDs, is expressed in the foetal brain and during the first days of postnatal development [26, 28]. There is a switch in RNA splicing after birth to produce adult tau isoforms. That switch correlates with a marked reduction in tau phosphorylation [28–33]. Furthermore, different neurons seem to have different tau isoforms. An example is illustrated by the hippocampal granule cells of dentate gyrus containing only tau protein that lacks exon 10 [2].

The contribution of the tau isoforms to the development of tauopathies is important as evidenced by the fact that several tau mutations responsible for FTDP-17 affect the splicing of exon 10, increasing the ratio of four-repeat with respect to three-repeat isoforms. This is mainly due to intronic mutations that result in the forced expression of exon 10 [34–38]. On the other hand, mutations resulting in the deletion of lysine-280 lead to reduced splicing of exon 10 [39], while the E342V mutation may affect the splicing of exons 2 and 3 [40]. This is in contrast to Pick's disease in which tau filaments are predominantly made up of isoforms with three repeats [41]. It is assumed that in AD no alteration in tau isoforms is observed. However, it has been reported that in affected areas, the amount of mRNAs of tau isoforms including exon 10 are significantly increased [42]. Therefore, an imbalance among tau isoforms and probably the type of neuron involved are relevant for the development of a specific tauopathy.

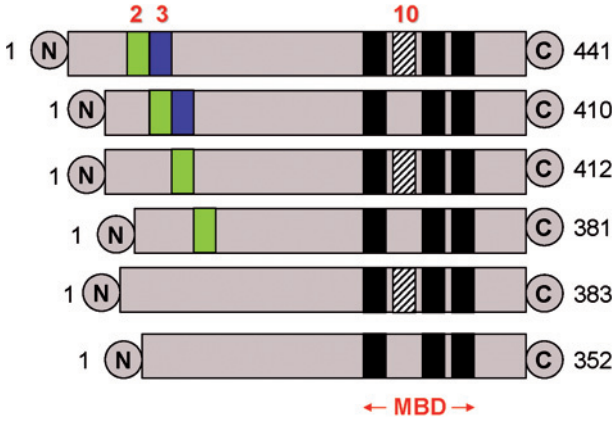

Figure 1. Tau expression. A single tau gene located on human chromosome 17 is transcribed into the corresponding nuclear RNA that, after alternative splicing, yields several tau messenger RNAs (mRNAs). These mRNAs, upon translation, originate the different tau isoforms. The complexity of the tau isoforms can then be increased by posttranslational modification, such as phosphorylation. A scheme of tau isoforms present in the central nervous system is indicated, showing the microtubule binding domains (MBD) on the tau molecule. The tubulin binding repeats are indicated in black (except exon 10, which is shown dashed) within the tau molecule. Exons 2 (green) at the amino-terminal region and exon 3 (blue) are also shown.

Taking into account its role in FTDP-17 pathology, splicing of exon 10 has been studied in detail. Mutations mapped around exon 10 lead to increased levels of tau isoforms containing exon 10. These include missense mutations that alter splicing enhancers and silencer regions [43–45] as well as mutations that alter the formation of an RNA stem-loop structure at the 5′ splice site of exon 10 [34]. Another level of regulation is phosphorylation. Thus, the selection of alternative splicing sites by SC-35 (an SR protein with an arginine- and serine-rich domain in its C-terminal end) is modulated by GSK-3β phosphorylation [46].

To add more complexity to the system, tau gene polymorphism has been described. Thus, two different tau gene haplotypes, H1 and H2, have been identified [47]. H1 is the most common, while the H2 haplotype

is only present in Caucasian populations [48]. The H1 haplotype, or at least some variants, is a risk factor for tau disorders like progressive supranuclear palsy and corticobasal degeneration [49–51]. Although the mechanism for disease susceptibility associated to H1 haplotype remains unknown, it has been suggested that, taking into account that the H1 promoter region seems to be more efficient than H2 at the transcriptional level, high levels of tau protein may be the basis for the risk factor associated to tau haplotype [52]. This idea is in agreement with the notion that some of the pathological aspects observed in transgenic mice overexpressing tau protein may be due to the high levels of tau protein obtained in murine models.

Degradation of tau protein

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 specific N-terminal residues, the PEST sequence and the destruction box must be considered [53]. Of the stabilization signals, amino acid repeats containing polyglutamine, glycine or alanine residues are among the most common [53]. PEST sequences are present in the N-terminal end of the tau molecule, while the MBD is a glycine-rich sequence. Little is known about the implication of these two regions in the stability of tau protein.

The existence of ubiquitin-independent proteosomal degradation of tau protein has been reported based on the fact that tau protein is degraded by the 20S proteasome in vitro [54]. However, there is strong evidences of tau degradation by the ubiquitin-proteasome system (UPS) after ubiquitylation of the protein. The ubiquitin-conjugating enzymes involved in the first steps of tau degradation by the UPS were characterized after purification of phosphorylated tau obtained from AD brain. That hyperphosphorylated tau binds to Hsc70. Its phosphorylation is necessary for the addition of ubiquitin by the E3 Ubiquitine ligase known by the acronym CHIP (carboxyl terminus of the Hsc70-interacting protein). Its C-terminal U box domain associates with the E2 protein-conjugating enzyme UbcH5B [55, 56]. As a consequence, deletion of CHIP in mice leads to the accumulation of hyperphosphorylated tau [57].

The notion that alterations in the UPS might play a role in tauopathies arises from the observation that in most of these diseases aberrant proteinaceous deposits can be detected inside the affected neurons using anti-ubiquitin and anti-proteasome antibodies [58, 59]. This hypothesis is supported by the previous observation that a significant decrease in proteasome activity was observed in AD patients [60]. Data to suggest that the UPS inhibition is involved in tauopathies are the following: (i) tau protein present in PHFs is ubiquitinated [61]; (ii) by doing tandem mass spectrometry, it has been observed that tau, isolated from PHF, is modified by ubiquitination, at lys 254, lys 311 and lys 353 [62]; (iii) immunohistochemistry studies have demonstrated that intracellular neurofibrillar tangles (NFTs) and pick bodies are CHIP- and ubiquitin-positive [56]; (iv) PHF-tau isolated from AD brain resulted in inhibition of proteasome activity [63].

The degradation of tau by different proteases has been studied. Thus, tau cleavage by caspases has been reported [64]. Tau is cleavaged by caspases at a highly conserved aspartate residue (Asp421) in its C terminus in vitro and in neurons treated with amyloid-beta (Abeta) (1–42) peptide generating a truncated protein that lacks its C-terminal 20 amino acids and assembles more rapidly and more extensively into tau filaments *in vitro* than wild-type tau [65]. These data suggest that activation of caspases and cleavage of tau in the AD brain may proceed to the formation of NFTs. Tau is also a substrate of the calcium-activated protease calpain [66], although phosphorylated tau is more resistant to proteolysis by calpain degradation than unphosphorylated tau.

Cathepsin D has been shown to cleave tau proteins, generating fragments similar to those found in NFT [67]. Interestingly, overexpresion of tau protein with FTDP-17 mutations in transgenic mice increased numbers of lysosomes displaying aberrant morphology similar to those found in AD [68]. Recently, a puromycin-sensitive aminopeptidase (PSA) was identified as a peptidase that proteolyzes tau protein [69]. PSA protected against tau-induced neurodegeneration in vivo, whereas PSA loss of function exacerbated neurodegeneration [69]. Finally, phosphorylated tau interacts with the protein HSP-27 [70]. HSP-27 is a protein that facilitates degradation of pathogenic hyperphosphorylated tau by an unknown mechanism.

Posttranslational modifications

Several modifications have been described for tau protein, including phosphorylation, glycosylation, ubiquitinylation, deamidation, oxidation, tyrosine nitration, cross-linking, glycation and truncation by protein cleavage [1]. However, the most studied of these has been serine/threonine phosphorylation. Tau is a phosphoprotein [11, 17, 71] with 79 putative serine or threonine phosphorylation sites on the longest central nervous system (CNS) tau isoform, which contains 441 residues (Fig. 1). These sites have been

divided into two main groups: those that can be modified by proline-directed kinases like tau protein kinase I (GSK3), tau protein kinase II (cdk5), MAP kinase (p38), JNK and other stress kinases or cdc2; and those that can be modified by non-proline-directed kinases like PKA, PKC, CaM kinase II, MARK kinases [72–79] or CKII, which modifies residues close to acidic residues mainly in exon 2 and 3 [74]. In the last year, new features of tau phosphorylation were reported. Thus, tau can be modified by a novel tau-tubulin kinase, member of the casein kinase I superfamily [80] that not only modifies tau residues 198, 199, 28 and 422 but also facilitates tau aggregation. Also, it has been reported that the phosphoepitope recognized by ab AT 100 could be raised by the sequential phosphorylation of tau by PKA and a stress kinase like p38 delta or JNK2 [81]. In addition, the roles for cdk5 and GSK3 on tau phosphorylation have been discussed, since cdk5 could act like a priming kinase for GSK3 [82], but it could also inhibit GSK3 activity [83].

In addition to serine/threonine modifications, phosphorylation at tau tyrosines has been also reported. The protein of the *src* family, *fyn*, modifies tyrosine 18 at early stages of development [84]. It has been suggested that missense mutations of tau, present in FTDP-17, could increase the binding of tau to fyn [85], facilitating the phosphorylation of tau by that kinase. Phosphorylation at other tyrosines has been also reported [86]. More recently, it was reported that not only fyn (which modifies tyr 394) can modify tau tyrosines but also *c-abl* [87]. On the other hand, other tyrosine modifications, such as nitration, take place at tyr 29 [88].

Several phosphatases, such an protein phosphatase (PP)1, PP2A, PP2B (calcineurin) and PP2C [89–93], are able to dephosphorylate tau protein. However, only PP1, PP2 and PP2B have been shown to dephosphorylate abnormally hyperphosphorylated tau [94, 95]. 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 [97, 98]. PP2A binds to tau through its tubulin binding region [99]. Mutations in that 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 [100]. A role for phosphatases in AD has been suggested following the observation of an increase in tau phosphorylation in hypothermia induced by reduced glucose metabolism in mice mainly because PP2A is less active than kinases in that condition [101]. 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 (for a review see [102]).

Inhibitors of tau phosphorylation

All these data raise the possibility of designing novel therapeutic interventions for AD and related tauopathies based on inhibiting tau kinase, especially GSK-3 [103]. Lithium, a widely used drug for affective disorders, inhibits GSK3 at therapeutically relevant concentrations [104]. Previous studies have suggested that lithium may be a useful drug for modulating tau hyperphosphorylation and neurodegeneration in AD [105]. First, lithium treatment has been demonstrated to inhibit tau phosphorylation both in cultured neuronal cells and in vivo in rat brain [106, 107]. Second, lithium also blocks tau hyperphosphorylation and inhibits cell death in cultured neurons treated with β-amyloid peptide [105]. Third, lithium seems to provide neuroprotection against a variety of toxic insults both in cultured neurons and in vivo in several animal models [108,109]. Finally, some evidence from brain imaging studies is consistent with the neurotrophic/neuroprotective effect of chronic lithium treatment in patients with bipolar mood disorder [110]. In addition, lithium has been demonstrated to have a beneficial effect on two FTDP-17 mouse models [111–113] as well as a mouse model that overexpresses the shorter human tau isoform [114]. AR-A014418 is a GSK-3 inhibitor [115] which is also able to inhibit tau phosphorylation in an FTDP-17 transgenic model [112]. In addition to GSK-3 inhibitors, other molecules have been tested to reduce tau phosphorylation. Some of these inhibitors are the kinase inhibitor K252a [116] and the octapeptide NAP [117]. The mechanism for other tau phosphorylation inhibitors, such an minocycline, remains to be further analyzed [118]. Alternatively, there are transduction pathways which modulate tau phosphorylation through a signalling cascade, like that of wnt [119]. On the other hand, the peroxisome proliferator-activated receptor-gamma decreases tau phosphorylation by affecting the cascade involving PDPK-1/p70S6kinase/mTor [120]. Also, agonists of alpha 7 nicotinic receptors decrease tau phosphorylation through the PI3kinase/PKB/GSK3 pathway [121].

Another way to regulate tau phosphorylation is to increase the activity of PP2A, the main tau phosphatase. The intracellular activity of PP2A is regulated by two proteins, I1-PP2A and I2-PP2A, and inhibition of

these proteins could result in an increase of phosphatase activity [122].

Does tau phosphorylation have any function?

It is well known that phosphorylated tau has a reduced affinity for microtubules and a reduced ability to promote microtubule assembly [123, 124]. This has also been proven both in *in vitro* assays and in transfected cells with tau phosphorylated by GSK-3 [125]. In good agreement, tau hyperphosphorylation in transgenic mice overexpressing GSK-3β shows accumulation of microtubule-unbound tau in hippocampal neurons [126, 127]. In addition, tau phosphorylation could affect axonal transport, since tau could bind to microtubules overlapping the sites used for kinesin protein motors [128]. Furthermore, tau-induced neurotoxicity in tauopathies has been related to the association of tau protein with actin, as tau is able to promote alterations in the actin cytoskeleton [129]. In fact, it has previously been reported that MBD is also involved in actin binding [130]. However, it is not yet clear whether tau phosphorylation could result in the gain of any unknown specific function or if it only alters the functions for unmodified tau. In this respect, aberrant intraneuronal protein deposits are a common feature for many neurodegenerative diseases, such as tauopathies. Whether these inclusion bodies are a common pathogenic mechanism for all of these diseases [131] or a protection mechanism as recently proposed for the intracellular aggregates in Huntington's disease [132] is still a matter of debate. The fact that transgenic mice overexpressing GSK-3β show impaired LTP [133] and cognitive impairment without the formation of tau filaments [134] argues against tau aggregates as a key pathogenic agent. In good agreement, it has been shown that cognitive deficits in mice that conditionally overexpress FTDP-17 human tau depend on transgene expression but not on tau filament formation [135]. Thus, these mice recovered spatial memory when silencing transgene expression but did not prevent the development of NFT.

Mechanism for pathological tau phosphorylation

Tau protein is aberrantly phosphorylated in tauopathies, and possible mechanism involved in that phosphorylation could be different for the different tauopathies. In the case of FTDP-17, mutations in the tau gene result in the onset of the disease [35]. Some of those tau mutations, as we have seen before, alter tau splicing, while others are missense mutations that occur in or around the MBD, decreasing the affinity of mutant tau by microtubules. As a consequence, unbound tau accumulates and is phosphorylated. This phenomenon is exacerbated as PP2A shows a decreased affinity for the mutated tau forms [100]. For the case of progressive supranuclear palsy, the generation of compounds such as hydroxynonenal (HNE) from lipid oxidation, could activate some stress kinases that could be involved in tau phosphorylation [136, 137].

Tau phosphorylation is also modulated by ApoEε4 isoform, a risk factor associated with the development of late-onset AD [138, 139]. ApoE through reelin receptors stimulates phosphatidylinositol 3-kinase and then protein kinase B/GSK-3β [140, 141]. More work has been done to analyze the possible mechanism for tau phosphorylation in familial Alzheimer's disease (FAD). Three genes have been found to be the cause of the onset of FAD. The first one that was described code for APP, the other two for two proteins known as presenilin-1 (PS-1) and presenilin-2 (PS-2). One of the functions of PS-1 and PS-2, the other proteins linked to FAD, is to facilitate the cleavage of APP, yielding β-amyloid [142, 143]. Additionally, it was found that aggregates of β-amyloid induce tau phosphorylation [144] and that GSK-3β (Tau kinase I) is essential for β-amyloid-induced neurotoxicity [145]. Also, it has been reported that Aβ mediates tau hyperphosphorylation by downregulation of PP2A [146]. These and other studies were the base of the so-called amyloid hypothesis [147, 148]. This hypothesis suggest that the origin of AD must be in the appearance of β-amyloid peptide. β-Amyloid production is facilitated by mutations associated to FAD in APP or PS (a gain of function). β-Amyloid will aggregate, those aggregates facilitate tau phosphorylation, and as consequence of that phosphorylation, tau protein polymerizses into PHF and later on aggregates into NFT. If that is the case, PS mutations yielding increasing amounts of β-amyloid peptide will induce a faster onset of FAD. However, that is not the case [149], as the amount of β-amyloid generated in cells transfected with different PS-1 mutations did not correlate with the starting age of FAD caused by that mutation. Supporting this view is a PS1 mutation in a patient with Pick-type tauopathy without extracellular β-amyloid deposits [150]. Thus, presenilin proteins could have another function that could be important for AD onset. One of these functions was described by Weihl et al. [151] indicating that PS-1 downregulates PKB. Subsequently, Baki et al. [152] reported that PS-1 activates PI3K, inhibiting GSK-3 activity and tau hyperphosphorylation. Therefore, it could be that some PS-1 mutations will result in a lack of PI3K activation (loss of function) that will lead to tau phosphorylation. In short, appear to be two pathways

from preselinin to induce AD. In one case, a gain of function will result in the appearance of β-amyloid and, afterwards, tau phosphorylation by GSK-3; in the other case, there will be loss of function, but the result is still tau phosphorylation by GSK-3. Thus, a common feature for FAD from PS-1 mutations will be the appearance of tau phosphorylated by GSK-3. More recently, it has been reported that PS-1 can control the leakage of calcium out of the endoplasmic reticulum [153], and that PS-1 mutations could result in an increase in cytoplasmic calcium that will activate tau kinases such as protein kinase C or calmodulin kinases.

On the other hand, in old people with old neurons, activation of NMDA receptors could activate ERK/MAPK-activated protein kinases that modify tau protein. Also, upon NMDA receptor activation, there is activation of the protease calpain, which degrades tau into a 17-kDa peptide, and a N-terminal peptide that could be toxic to the cell [154, 155]. Finally, changes in the pathways involving wnt [156] or insulin [101] could result in abnormal tau phosphorylation. Tau undergoes reversible hyperphosphorylation when a mouse is starved for 2 days [157] or forced to swim in cold water [158]. Since starvation and swimming in cold water result in the elevation of corticosterone levels in rodents [159], it has been suggested that tau phosphorylation could be a consequence of a neuronal stress reaction Also, changes in hibernation [160] could modify the level of tau phosphorylation. On the other hand, learning reduces tau phosphorylation [161].

Aggregation

A main feature of the different tauopathies is the presence of aberrant tau aggregates, a characteristic that could be reproduced in vitro [1]. Purified tau protein can form fibrillar polymers resembling the PHF found in the brain of AD patients [14, 162–165]. It has been shown that a high concentration of protein is needed for tau to polymerize [162], 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 [166, 167]. Free fatty acids may also facilitate tau aggregation [165]. It is noteworthy that lipid peroxidation occurs in AD, and a compound such as arachidonic acid could be fragmented to yield toxic products like HNE; it has been shown that HNE facilitates tau assembly [168, 169]. Finally, some quinones could also induce tau polymerization in fibrillar form [170, 171].

Analysis of the minimal region of tau protein involved in self-aggregation indicates that the residues comprising the third tubulin binding repeat in the tau molecule are needed for self-assembly [167]. Moreover, a hexapeptide comprising residues 306–311 in tau molecule has been suggested to be the minimal region involved in tau polymerization [172].

Although the number of mRNAs of tau isoforms, including exon 10, seems to be significantly increased in AD-affected areas [42], aggregation of tau in AD does not depend on tau isoforms [173]. Rather, particular phosphorylation profiles may regulate the aggregation of tau into PHF. Thus, the role of phosphorylation in the self-assembly of tau is a fundamental question in the study of AD and other tauopathies. It has been suggested that phosphorylation of some specific tau sites may be a prerequisite for its assembly [174, 175]. Some of these sites occur, indeed, in the PHF core-forming MBD domain. GSK-3β is one of the best candidate enzymes for generating the hyperphosphorylated tau that is characteristic of PHFs (for a review, see [176]). However, in vitro experiments to test the effect of phosphorylation on tau self-assembly have shown varied results, presumably reflecting slight differences in experimental conditions. In some experiments phosphorylated tau displays a decreased propensity to aggregate [177], whereas in other conditions the aggregation propensity increases [168]. Thus, discrepancy in the results can be due to differences in tau protein concentration or in the status of tau phosphorylation. In fact, phosphorylation of some regions can probably inhibit aggregation, while phosphorylation of other regions can induce tau polymerization. Compounds such as HNE [168] and several quinones [170] catalyze the formation of fibrillar aggregates of phosphorylated tau peptides but fail to have an observable effect if the peptides are in a non-phosphorylated form. Another fundamental question refers to the molecular structure of tau protein in the PHF particle. The current view is that pathological protein aggregation must involve formation of β-sheet structure giving rise to the typical amyloid fibrils [178]. The discovery of α-helix structure in *ex vivo* PHFs [179] indicates that PHFs are not a typical case of amyloidosis. The role of α-helix structure in PHF formation is also supported by recent work showing that the region implicated in forming the core of PHFs (i.e., MBD domain) becomes very α-helix upon addition of the helix-promoting agent TFE [180, 181]. The fact that phosphorylation of tau occurs in sites of the same region that is involved in forming the core of PHFs has led to suggestion that phosphorylation controls assembly. However, no mechanistic model of the interplay between phosphorylation and assembly has been

proposed for full-length tau. This issue has been addressed using a peptide corresponding to tau region 317–335 as the simplest model system. Results revealed a higher helical propensity of region 317–335 upon phosphorylation [182].

A number of mouse transgenic models have been developed in recent years with the objective of reproducing aspects of tau pathology as found in AD and in tauopathies. 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 [183]. Human tau is distributed in neurites and at synapses but is absent from cell bodies, and no neuropathological lesions were reported in mice up to 8 months of age. However, overexpression of human tau in mice in the absence of mouse tau results in an aberrant tau aggregation [184]. A pretangle tau pathology has been reported in mice overexpressing the shortest human tau isoforms (151, 152). An analogous accumulation of hyperphosphorylated tau in a somatodendritic compartmentalization in hippocampal neurons has also been observed in a mouse transgenic model overexpressing the tau-phosphorylating enzyme GSK3, with the conditional expression of the transgene after the development of the CNS [126].

Transgenic mice containing the FTDP-17 mutated P301L have been characterized [185], as well as one containing the P301S mutation [186]. In the latter, abundant tau filaments within a PHF structure 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 [187]. Filaments were also found in transgenic mice expressing the mutation R406W in human tau [188], a mutation that decreases the phosphorylation of tau at the site recognized by Ab PHF-1 [189]. Finally, it has been shown that filaments form in transgenic mice expressing mutant (V337M) human tau [190]. Another approach consists in generating transgenic mice with several FTDP-17 mutations (G272V, P301L and R406W, [68]). Ultrastructural analysis of these mutant tau mice revealed pre-filaments of tau as well as an increased numbers of lysosomes displaying aberrant morphology similar to those found in AD [68]. Filaments similar in width to those found in tauopathies were found after crossing that mouse model with transgenic mice overexpressing the enzyme GSK-3β [113].

On the other hand, expression of other mutation proteins like PS-1 could favour the formation of tau inclusions in mice [191]. Finally, aggregation of tau has been also induced in cell models [169, 192, 193] and in organotypic hippocampal slices [194].

Tau toxicity

The development of tau pathology associated with AD has been described by Braak and Braak [195] by following the development of neurofibrillary lesions at different stages of the disease. In the hippocampus, an inverse relation has been found between the number of extracellular NFTs and the number of surviving neurons [196–199]. It suggests that neurons that degenerate have previously developed tau aggregates. Also, it has been reported that the severity of dementia correlates with deposition of NFT in AD [200]. On the other hand, it has been suggested that neurons bearing neurofibrillary lesions could survive for a long period of time [201], and comparison with other neurodegenerative disorders, like Huntington disease [132], indicates that tau aggregates could protect against neurodegeneration by sequestering toxic (phospho?) monomeric tau molecules that could be present in a high amount inside a cell in pathological conditions. It has also been suggested, using a transgenic mouse model [135], that memory deficits could be unrelated to the formation of tau polymers, although more recently the discussion of those experiments suggested that hyperphosphorylated, aggregated tau intermediates could be the ones that cause neurodegeneration [202]. In this way, the implication of different types of protein aggregates in neurodegeneration has been extensively discussed [200]. A possibility of the existence of neurotoxic tau intermediate aggregates in human tauopathies is based on the fact that patients with FTDP-17 show extensive neurodegeneration with a high level of tau phosphorylation but with a low number of tangles [203]. In any case, even if the formation of tau aggregates has a protective function for the neurons, that function is not working well, as described by Braak and Braak [195], and subsequently by Delacourte et al. [204], indicating a correlation between progression of tau pathology and progression of the disease. This idea is supported by experiments indicating that neural loss and neurofibrillary tangle number increase in parallel with progression of the disease [205]. Similar results have been reported in other neurological disorders such as brain encephalopathies, where formation of aberrant polymers is related to the onset of neurodegeneration [206].

NFT seems to be a very stable structure. Thus, different treatments revert soluble hyperphosphorylated tau, but do not change back already formed NFTs (Fig. 2). That has been observed after administration of GSK-3 inhibitors [207] and by active and passive immunization against β-amyloid [208]. The same has been reported in transgenic mice overexpressing FTDP-17 tau in a conditional

model, and after turning out the system with doxicycline [135].

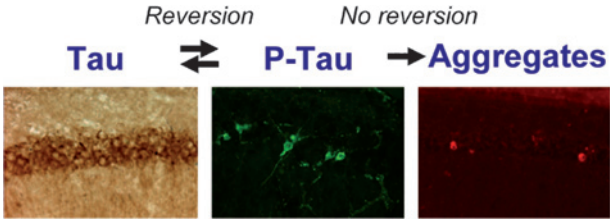

Figure 2. The development of tau pathology is characterized by an increase in phospho-tau and by the presence of NFT. Here is shown tau pathology in a transgenic mouse overexpressing GSK-3β and FTDP-17 tau [127, 207]. Transgenic tau accumulates in the somatodendritic compartment, but only in old mice the number of phospho-tau-positive neurons increase (center panel). Immunohistochemistry carried out with T14 antibody, which recognizes only human transgenic tau (left panel), and immunoflorescence carried out with the phospho-tau antibody AT8 (center panels) is shown for double transgenic mice. The right panel shows Thiazin Red-positive neurons (NFT-like structures) that were found in hippocampal pyramidal neurons only at the age of 18 months. It is still possible to partially reverse tau pathology in advanced stages of the disease. Thus, when lithium (a GSK-3 inhibitor) is administered to these mice, phosphorylated tau decreases, although tau aggregated in NFT-like structures does not revert to its previous state [207]. NFT seems to be a very stable structure. Thus, other different treatments (see text) revert soluble hyperphosphorylated tau, but do not change back already formed tau aggregates [135, 208].

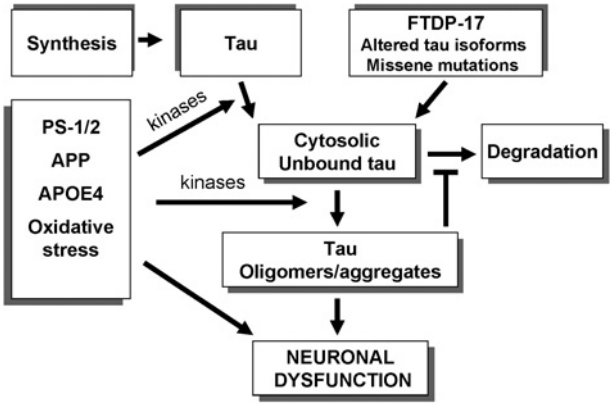

Figure 3. A model for neuronal dysfunction in tauopathies. Transduction pathways altered in familial Alzheimer's disease (APP and PS-1) induces tau phosphorylation. Furthermore, risk factors associated with the development of late onset of Alzheimer's disease such as the isoform ApoEε4 as well as pathological conditions as oxidative stress are also associated with tau phosphorylation. Phosphorylated tau has less affinity for microtubules and accumulates in the somatodendritic compartment. In FTDP-17, tau mutations as well as those that alter tau isoforms decrease the affinity of mutated tau by the microtubules. Unbinding of tau from the microtubules may result in its hyperphosphorylation and, subsequently, in its assembly into polymers like PHFs. Phosphorylated tau can be assembled in the presence of compounds such as hydroxynonenal (HNE), a molecule that results from oxidation of fatty acids. Although these tau aggregates could protect neurons against toxic hyperphosphorylated microaggregates, after a period of survival neuronal death could occur. Furthermore, tau aggregates can inhibit the proteasome.

Summary

A relationship between the expression, structure, phosphorylation, aggregation, and toxicity of tau is not yet clear. Pathologies in which tau is implicated could initially be the result of the presence of hyperphosphorylated tau in the somatodendritic compartment. As we have analyzed, most of the transduction pathways altered in FAD disease (APP and PS-1) induce tau phosphorylation. Furthermore, risk factors associated with the development of late onset of Alzheimer's disease, such as H1 tau haplotype and the isoform ApoEε4, are also associated with tau phosphorylation. Furthermore, pathological conditions associated with tauopathies such as oxidative stress and alterations in glucose metabolism also induce tau phosphorylation. That hyperphosphorylation could be maintained if phosphatases like PP2A were not functioning properly. Hhyperphosphorylated tau could then be assembled into PHFs, in the presence of other compounds that might facilitate polymerization (Fig. 3). Although these tau aggregates could protect neurons against toxic hyperphosphorylated microaggregates, after a period of survival [209] neuronal death could occur, followed by cell lysis that would liberate tau into the extracellular space where it could induce death [210]. Finally, the development of possible therapies against tau pathology will be commented in an additional review in this issue of CMLS.

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