Brain Pathology 9: 681-693(1999)

SYMPOSIUM: Tau and Synuclein in Neuropathology

Comparative Biochemistry of Tau in Progressive Supranuclear Palsy, Corticobasal Degeneration, FTDP-17 and Pick's Disease

Luc Buée and André Delacourte

INSERM U422, F-59045 Lille, France

Neurodegenerative disorders referred to as tauopathies have cellular hyperphosphorylated tau protein aggregates in the absence of amyloid deposits. Comparative biochemistry of tau aggregates shows that they differ in both phosphorylation and content of tau isoforms. The six tau isoforms found in human brain contain either three (3R) or four microtubule-binding domains (4R). In Alzheimer's disease, all six tau isoforms are abnormally phosphorylated and aggregate into paired helical filaments. They are detected by immunoblotting as a major tau triplet (tau55, 64 and 69). In corticobasal degeneration and progressive supranuclear palsy, only 4R-tau isoforms aggregate into twisted and straight filaments respectively. They appear as a major tau doublet (tau64 and 69). Finally, in Pick's disease, only 3R-tau isoforms aggregate into random coiled filaments. They are characterized by another major tau doublet (tau55 and 64). These differences in tau isoforms may be related to either the degeneration of particular cell populations in a given disorder or aberrant cell trafficking of particular tau isoforms. Finally, recent findings provide a direct link between a genetic defect in tau and its abnormal aggregation into filaments in fronto-temporal dementia with Parkinsonism linked to chromosome 17, demonstrating that tau aggregation is sufficient for nerve cell degeneration. Thus, tau mutations and polymorphisms may also be instrumental in many neurodegenerative disorders.

Introduction

The cytoskeleton plays a major role in establishing and maintaining the regional specialization within neurons. Microtubules, polymers made of tubulin, are responsible for neurite extension and serve as the tracks for transport within the cells. Microtubule-associated proteins also play important roles in the assembly of microtubules, in cross-linking of microtubules to each other and to other filaments, and in transport functions. In some neurodegenerative disorders, referred to as tauopathies, hyperphosphorylated microtubule-associated tau protein aggregates into abnormal filaments. These filaments are found in glial and neurofibrillary tangles, degenerating neurites and Pick bodies. Some tau aggregates are consistently found in Alzheimer's disease (AD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and some frontotemporal dementia including fronto-temporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) and Pick's disease (PiD) (2, 31, 46).

Corresponding author:
Luc Buée, INSERM U422, Place de Verdun, F-59045 Lille Cedex, France; Tel.: 33 / (0) 320 622074; Fax: 33 / (0) 320 622079; E-mail: buee@lille.inserm.fr

Tau proteins

Tau belongs to the family of microtubule-associated proteins (131) and is involved in microtubule assembly and stabilization. In humans, tau is found in neurons, although non-neuronal cells also have trace amounts (52). In the adult human brain, six tau isoforms are produced from a single gene, located on chromosome 17q21, by alternative mRNA splicing. Exons 2, 3 and 10 are alternatively spliced and allow for six combinations (2-3-10-; 2+3-10-; 2+3+10-; 2-3-10+; 2+3-10+; 2+3+10+) (42, 43, 75).

At the protein level, tau proteins constitute a family of six isoforms ranging from 352 to 441 amino acids with molecular weights from 45 to 65 kDa, when run on SDS-PAGE (Figure 1). The tau variants differ from each other by the presence or absence of 29- or 58-amino acids inserts located in the amino-terminal part and a 31-amino acids repeat located in the carboxy-terminal part. In absence of the latter, which is encoded by exon 10, the spliced products give rise to three tau isoforms with three repeats (3R). The three other tau isoforms contain this 31 amino acids repeat and thus have four repeats (4R). These repeats and their adjacent domains constitute the microtubule-binding domains of tau (42,

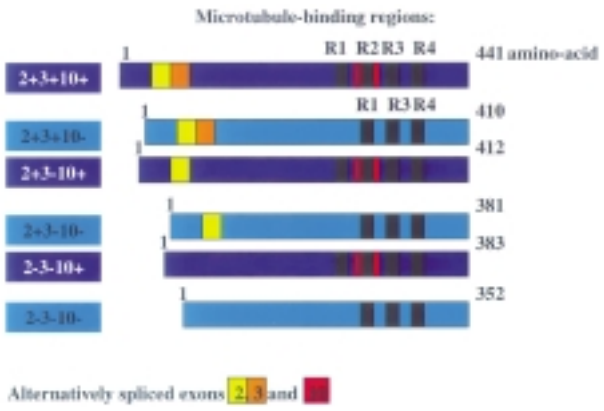

Figure 1. Schematic representation of the six brain tau isoforms (ranging from 352 to 441 amino-acid). Alternative splicing of the exons 2, 3 and 10 allows the six combinations (2-3-10-; 2+3-10-; 2+3+10-; 2-3-10+; 2+3-10+;2+3+10+). They differ from each other by the addition of one or two 29 amino-acid inserts (encoded by exons 2 (yellow) and 3 (orange) in the amino-terminal domain in combination with either three (R1, R3 and R4) or four (R1-R4) microtubule-binding domains (referred to as 3R (light blue) and 4R (dark blue) respectively). The fourth microtubule-binding domain (R2) is encoded by exon 10 (red box).

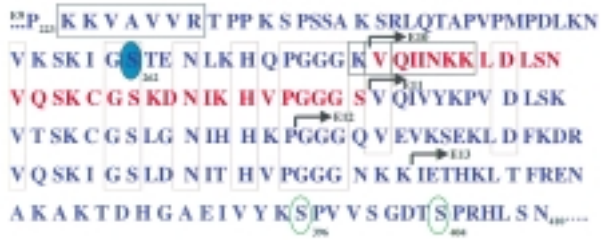

Figure 2. Partial sequence of the 441 amino-acid tau isoform (Pro223-Asn410) including microtubule-binding domains. Consensus sequences among the four microtubule-binding domains are gray-boxed. Other major sequences with microtubule-binding properties are the heptapeptide in exon 9 (blue box), R1-R2 inter-repeat (blue box) and the phosphorylation site Ser262 (dark green circle). The sequence encoded by exon 10 is in red. Beginning of the sequences encoded by exons 10, 11, 12 and 13 are indicated by an arrow. Ser396 and 404 are also indicated (light green circle).

43, 77, 78). In normal cerebral cortex, 3R-tau isoforms are slightly more predominant than 4R-tau isoforms (43). Furthermore, the two tau isoforms with the 58-amino acids insert are weakly expressed (64, 85) (Figure 1). Finally, tau isoforms may be differentially distributed in neuronal subpopulations. For instance, 4R-tau isoforms are not detected by *in situ* hybridization in granular cell of the dentate gyrus (43). These variations indicate that the different domains of tau are likely to be involved in various physiological functions.

There are 80 Ser or Thr residues in the longest human brain tau isoform (441 amino acids) and tau proteins can be phosphorylated at a number of these sites, some of which regulate their microtubule-binding properties. Using phosphorylation-dependent anti-tau antibodies, mass spectrometry and sequencing, at least thirty phosphorylation sites have been described (Table 1) (13, 54, 55, 68, 83, 91, 109, 125). All of these sites are localized outside the microtubule-binding domains with the exception of Ser 262 (R1), Ser285 (between R1 and R2), Ser305 (between R2 and R3), Ser324 (R3), Ser352 (R4) and Ser356 (R4) (42, 43, 109, 117). Most of these phosphorylation sites are on Ser-Pro and Thr-Pro motives. A number of sites on non Ser/Thr-Pro sites have also been identified.

Phosphorylated tau sites	Antibodies
T181	AT270
S202/T205	AT8
T212/S214	*AT100*
T231/S235	AT180
T231/S235	*PHF-27/TG3*
S262	12E8
S396/S404	AD2/PHF1
S422	*988/AP422*

Numbering is given according to the 441 amino-acid tau isoform. Antibodies that recognize abnormal tau phosphorylation are in italic. They are likely to recognize conformational epitopes.

Table 1. Phosphorylation-dependent monoclonal antibodies and their epitopes.

As indicated above, the carboxy-terminal part of tau proteins is characterized by the presence of 3 or 4 microtubule-binding domains. These repetitive domains are the repeats encoded by exons 9-12 (Figure 2). The 3R or 4R are made of a highly conserved 18-amino acids repeat separated from each other by less conserved 13- or 14- amino acids inter-repeat domains. It has been demonstrated that adult 4R tau isoforms are more efficient at promoting microtubule assembly than 3R tau isoforms. The R1-R2 inter-repeat is likely to enhance this binding (48). A heptapeptide (K_{224}KVAVVR$_{230}$) located in the proline-rich region has also a high microtubule binding activity in combination with the repeat regions (Figure 2) (48). However, microtubule assembly also depends partially upon the phosphorylation state of tau proteins: phosphorylated tau proteins are less effective than non-phosphorylated tau on microtubule polymerization (5, 8, 20, 21, 32, 80). Phosphorylation of Ser 262 alone dramatically reduces the affinity of tau for microtubules in vitro (5). Nevertheless, this site alone is

insufficient to eliminate tau binding to microtubules (117). Thus, phosphorylation outside the microtubule-binding domains can also influence tubulin assembly by modifying tau-microtubule affinity (48, 87).

Alzheimer's disease

Phosphorylation modifies tau biochemical properties, in that they become longer and stiffer (53). In neurodegenerative disorders, hyperphosphorylated tau proteins aggregate into intracellular filamentous inclusions. In AD, these filaments are named paired helical filaments (PHF). The major antigenic components of PHF are tau proteins (9), and several groups have reported phosphorylation as the major modification in these proteins (38, 49, 51, 66). Their biochemical characterization by SDS-PAGE and immunoblotting reveals the presence of a triplet of proteins at 55, 64 and 69 kDa (tau55, 64 and 69), and also referred to as A68, or PHF-tau (25, 44, 49, 79). A 72-74 kDa component is also present in only very low amounts (114). Using PHF-tau preparations and recombinant tau proteins, Goedert and colleagues showed that dephosphorylated PHF-tau proteins have a similar electrophoretic mobility than the six tau isoforms expressed in human brain (44). The following scheme is now well established (Figure 3): tau 55 results from the phosphorylation of the shortest isoform (2-, 3-, 10-); tau 64 from the phosphorylation of tau variants with one cassette exon (2+, 3-, 10- and/or 2-, 3-, 10+); tau 69 from the phosphorylation of tau variants with two cassette exons (2+, 3+, 10- and/or 2+, 3-, 10+). Phosphorylation of the longest tau isoform (2+, 3+, 10+) induces the formation of the additional hyperphosphorylated tau74 variant (85, 93, 113, 114).

Despite the fact that many phosphorylation sites are common to aggregated tau proteins, referred to as PHF-tau in AD, and native tau in control biopsy-derived materials, there are biochemical differences that differentiate them and support the concept of abnormal phosphorylation in AD (88, 113). First, insoluble polymers of phosphorylated tau are present exclusively in AD brain extracts and are visualized by immunoblotting as smears using anti-tau antibodies. Second, two-dimensional immunoblot analysis reveals that PHF-tau are more acidic than native tau derived from biopsy samples (113). Third, hyperphosphorylation generates differences that can be visualized by a few phosphorylation-dependent antibodies such as AT100 (86, 88, 133), AP422 (56), 988 (17), PHF-27 (63) and the TG/MC antibodies (i.e. TG3) (130). (Figure 4) With the exception of ser422, these sites in PHF-tau are conformation-dependent epitopes. Recently, it was also shown that

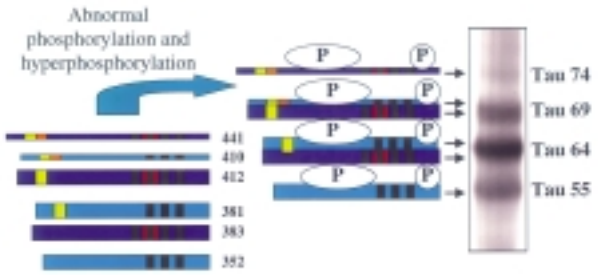

Figure 3. Schematic representation of the hyperphosphorylation (P blue-circled) of the six brain tau isoforms in Alzheimer's disease. The two tau isoforms with the 58-amino-acid insert are weakly expressed and are represented thinner than the others. Tau 55 results from the phosphorylation of the shortest isoform (2-, 3-, 10-), tau 64 from the phosphorylation of tau variants with one cassette exon (2+, 3-, 10- and/or 2-, 3-, 10+), tau 69 from the phosphorylation of tau variants with two cassette exons (2+, 3+, 10- and/or 2+, 3-, 10+). Phosphorylation of the longest tau isoform (2+, 3+, 10+) induces the formation of the additional hyperphosphorylated tau74 variant. The color codes are similar to those used in Figure 1. On the left, a typical immunoblotting using the phosphorylation-dependent monoclonal antibody AD2, that recognizes phosphorylated Ser396 and 404, allows to visualize the Alzheimer-type electrophoretic profile (tau55, 64 and 69 and the minor tau74 variant).

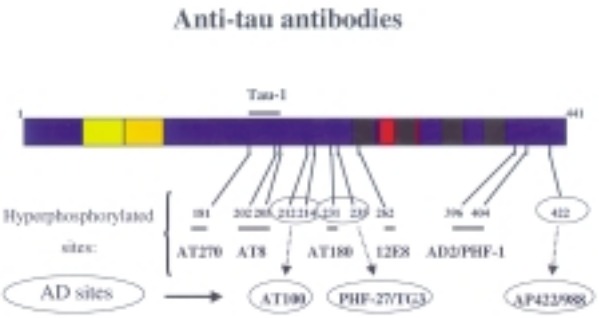

Figure 4. The binding sites of anti-tau antibodies. The different well-known antibodies and their binding sites are represented on the schematic map of the 441 amino-acid tau isoform (color codes are similar to those described in Figure 1). With the exception of Tau-1 that recognizes the dephosphorylated 189-207 amino-acid sequence, all antibodies bind to phosphorylated epitopes. Antibodies that recognize abnormal tau phosphorylation are blue-circled.

TG3 epitope was selectively expressed in mitotic cells, but not in quiescent cells (130). These data suggest that cell cycle mechanisms may be affected in AD and lead to neurodegeneration (74, 84, 130).

Altogether, these results show that the main feature of PHF-tau is their aggregation into polymers that constitute neurofibrillary lesions. The aggregation process may be enhanced by a number of co-factors as suggest-

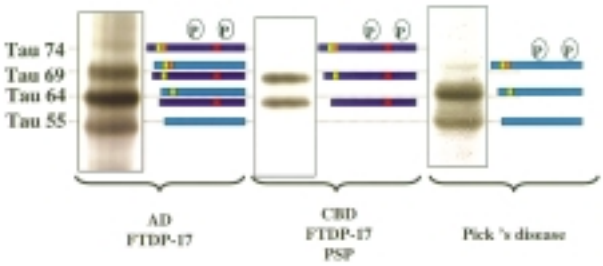

Figure 5. Typical western-blots using the phosphorylation-dependent monoclonal antibody AD2 exhibiting the electrophoretic tau profiles encountered in AD, CBD, FTDP-17, PiD and PSP. On the right side of each blot, the type of hyperphosphorylated tau isoforms that are found aggregated in filaments are represented. Color codes are similar to those used in Figure 1.

ed in amyloidosis. Among them, glycosaminoglycans and other polyanions might be of particular interest (41, 45, 69, 98, 120). In addition, and possibly in association with the aggregation process, specific phosphorylation sites are also present on PHF-tau. Tau aggregation is not specific to AD, and is also described in many other neurodegenerative disorders. Interestingly, the tau electrophoretic profile is often disease-specific.

Progressive supranuclear palsy

Progressive supranuclear palsy (PSP) is a cause of late-onset atypical Parkinsonism described by Steele, Richardson, and Olszewski in 1964 (121). Dementia is also a common feature at the end-stage of the disease (81, 82). Neuropathologically, PSP is characterized by neuronal loss, gliosis and NFT formation. Neurofibrillary tangles were first described in basal ganglia, brain stem, and cerebellum (121). Subsequently, neuronal degeneration was described in the perirhinal, inferior temporal and prefrontal cortex, with the same features as subcortical NFT (4, 59, 61). Furthermore, glial fibrillary tangles have also been described (4, 18, 59, 72, 73). Ultrastructural analyses further support differences between AD and PSP, since PHF are found in AD (71), while straight filaments are observed in PSP (123, 124).

The electrophoretic profile of aggregated tau proteins in PSP is substantially different from that in AD, as a characteristic doublet is found (Tau 64 and Tau 69) instead of the triplet of AD (40, 127). A minor 74 kDa band is also detected. In fact, only hyperphosphorylated tau isoforms with sequence encoded by exon10 (4R-tau isoforms) aggregate into filaments in PSP whereas tau isoforms without exon 10 (3R-tau isoforms) are not detected (85, 116) (Figure 5). Nevertheless, most of the phosphorylation sites in PHF-tau are also encountered in aggregated tau proteins from PSP patients (111). Biochemical mapping performed on several cortical and subcortical areas from PSP brain has revealed that the doublet of tau 64 and 69 is first detected in the subcortical regions where NFT are found, neocortical areas being affected later (127, 129). These results are in good agreement with previous neuropathological results that show cortical involvement in these areas in advanced disease (59, 61).

Although most cases of PSP are considered to be sporadic, familial cases such as those reported by De Yebenes and coworkers have a pattern of inheritance consistent with an autosomal dominant disorder (24). More recently, a study of clinical genetics of familial PSP suggests that hereditary PSP is more frequent than previously thought and that the scarcity of familial cases may be related to the lack of recognition of the variable phenotypic expression of the disease (110). Conrad *et al*. first identified a polymorphic dinucleotide repeat sequence in the intron 9 (between exon 9 and exon 10) of the tau gene, in a Caucasian population with PSP (22). They described a significant over representation of the most common allele (A0), characterized by the presence of 11 TG repeat, and of the homozygous genotype A0/A0 in the PSP cohort (95.5%), compared to normal controls (57.4%) or patients with AD (49.7%). Recently, these data were subsequently confirmed by several studies considering Caucasian series (3, 60, 92, 96). Conversely, it was not observed in Japanese populations (23). Moreover, Baker *et al*. (1999) described two extended haplotypes that cover the gene (2). In unrelated Caucasians, there was complete disequilibrium between polymorphisms that span the gene. These authors showed that the most common haplotype, designated H1, is significantly over represented in patients with PSP, extending earlier reports of the association between the intronic dinucleotide polymorphism and the disorder (2). While not likely to be directly involved in splicing given the distance from the splice site, it is interesting to speculate that the dinucleotide polymorphism influences in some way exon 10 splicing and, thus, the proportion of 4R-tau isoforms. Even if polymorphisms in the tau gene are important to the pathogenesis of PSP, it remains to be determined at what level it is involved. It is noteworthy that in some familial forms of PSP, no linkage to chromosome 17 is observed (110).

Corticobasal degeneration

Corticobasal degeneration (CBD) was first described

in 1967 by Rebiez and coworkers as corticodentatonigral degeneration with neuronal achromasia (103, 104). CBD is a rare, sporadic and slowly progressive late-onset neurodegenerative disorder that is clinically characterized by cognitive disturbances and extrapyramidal motor dysfunction (104). Moderate dementia emerges sometimes late in the course of the disease (107). There is a clinical and pathological overlap between PSP and corticobasal degeneration (37, 81, 82, 112). Neuropathological examination reveals glial and neuronal abnormalities. The glial pathology includes astrocytic plaques and numerous tau-immunoreactive inclusions in the white matter. Achromatic ballooned neurons are detected in cortex, brainstem and subcortical structures, as are neuritic changes and NFT. These lesions can be readily visualized with phosphorylation-dependent anti-tau antibodies (14, 36, 37, 73, 76, 97). Ultrastructural studies indicate that tau aggregates in CBD form twisted filaments that differ from PHF of AD. In CBD, filaments are shorter in length (less than 400 nm), 10 to 20% wider and the periodic twist (169 to 202 nm) is twice as long as that in AD (76).

The electrophoretic profile of tau pathological proteins in CBD is similar from that of PSP (14, 36, 76), and is described as a major tau 64, 69 doublet (Figure 5). The components may be different since this doublet is not detected in CBD using antibodies raised against the region encoded by exon 3 (76). These data have been confirmed by immunohistochemistry (36). Conversely, in recent studies, tau isoforms with sequence encoded by exon10 (4R tau isoforms) were found solely in CBD, whereas tau isoforms without exon 10 were not detected. These data suggest that only 4R-tau isoforms aggregate into filaments in CBD as observed in PSP (85, 116). In this respect, the only isoform with sequence encoded by both exons 3 and 10 is the longest tau isoform (Figure 5). Since the longest tau isoform is found in very low amounts in human brain, it may explain why previous works did not find any immunoreactivity of sequence encoded by exon 3 in their experiments (36, 76). These data confirm our observations that both size and phosphorylation of tau isoforms are responsible for the observed differences in tau electrophoretic mobility. It should be noted that to date no tau polymorphism has been reported in CBD

Pick's disease

Pick's disease is a rare neurodegenerative disorder characterized by a progressive dementia and personality deterioration. Early in the clinical course, patients often show signs of frontal disinhibition (11, 100).

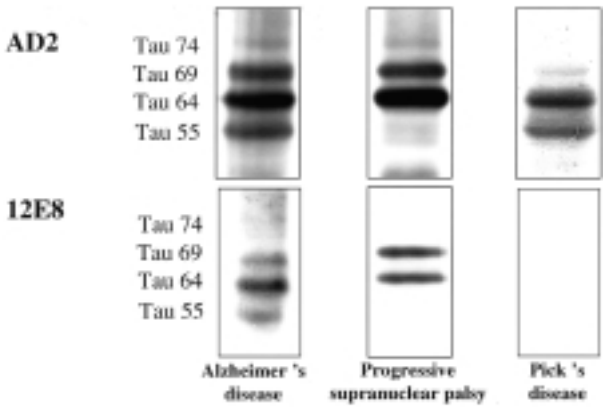

Figure 6. Typical western-blots using the phosphorylation-dependent monoclonal antibodies AD2 and 12E8. AD2 labels all tau variants among neurodegenerative disorders. The 12E8 antibody does not label the tau doublet tau 55 and 64 in PiD whereas it labels both the PSP major tau doublet (tau64 and 69) and the AD major tau triplet (tau 55, 64 and 69).

Tau mutations	Exon	Tau pathology
K257T	9	AD triplet ?
I260V	9	AD triplet ?
G272V	9	AD triplet
N279K	10	PSP doublet
ΔK280	10	?
L284L	10	PSP doublet
P301L	10	PSP doublet
P301S	10	PSP doublet
S305N	10	PSP doublet
+3	intronic	PSP doublet
+12	intronic	PSP doublet
+13	intronic	PSP doublet
+14	intronic	PSP doublet
+16	intronic	PSP doublet
V337M	12	AD triplet
G389R	13	AD triplet
R406W	13	AD triplet

Table 2.

Neuropathologically, Pick's disease is characterized by prominent frontotemporal lobar atrophy, gliosis, severe neuronal loss, ballooned neurons and the presence of neuronal inclusions called Pick bodies (11, 14, 26, 62). Pick bodies are immunolabeled by anti-PHF-tau antibodies, with a higher density in the hippocampus than in the neocortex (14, 26, 62). The laminar distribution of Pick bodies is clearly different from that of NFT in AD, CBD and PSP. In the hippocampus, Pick bodies are

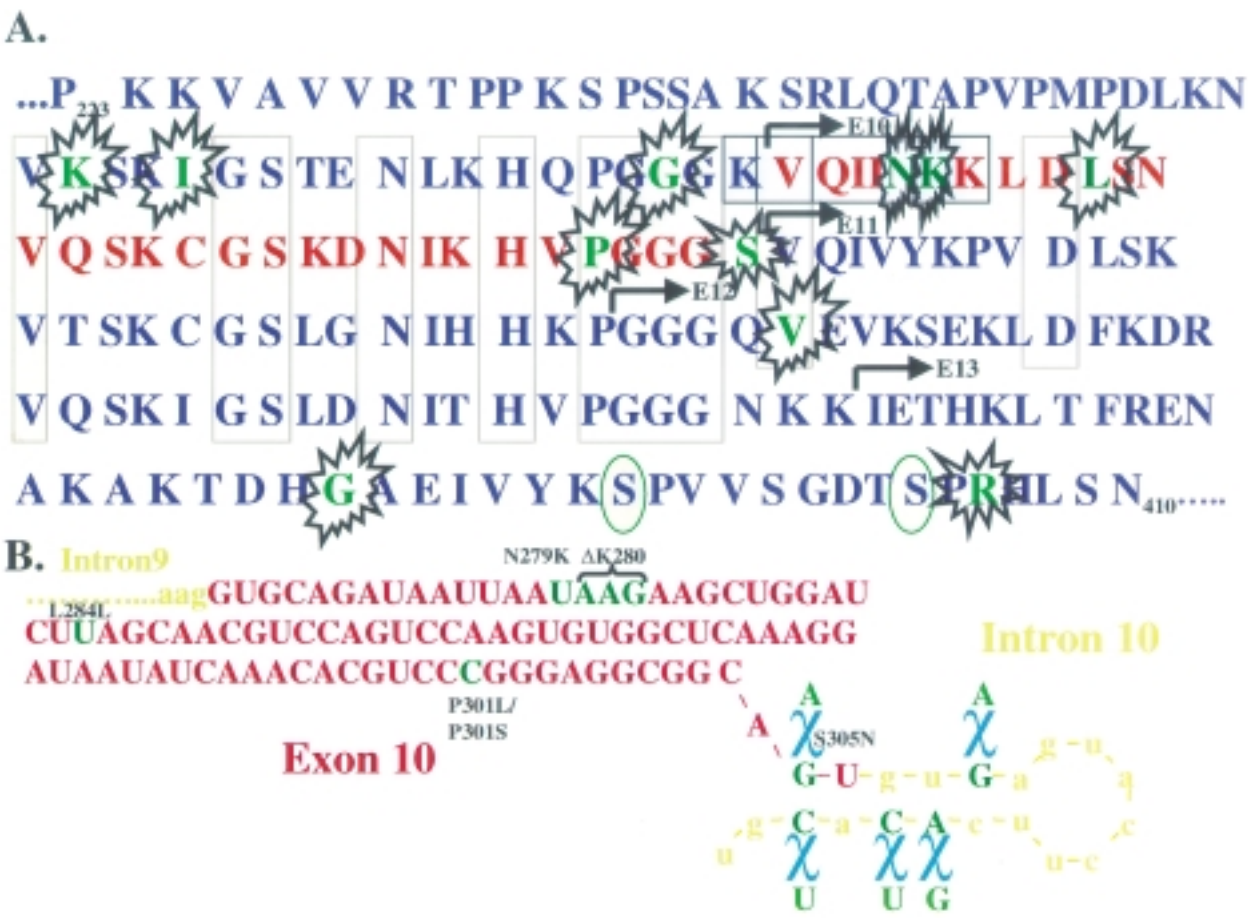

Figure 7. A. Partial sequence of the 441 amino-acid tau isoform (Pro223-Asn410) showing FTDP-17 mutations. Consensus sequences among the four microtubule-binding domains are gray-boxed. The heptapeptide with microtubule-binding properties in exon 9 is blue boxed. The sequence encoded by exon 10 is in red. Beginning of the sequences encoded by exons 10, 11, 12 and 13 are indicated by an arrow. Ser396 and 404 are also indicated (light green circle). All FTDP-17 mutated amino acids are in green in an explosion scheme. **B.** Nucleotidic sequence of the exon 10 and its 5' and 3' intronic regions. All FTDP-17 mutated nucleotides are in green. The exon 10 sequence is in red caps letters. The intronic sequence is in light brown. Mutations are only shown in the stem loop structure.

numerous in granular cell neurons of the dentate gyrus, in CA1, subiculum and entorhinal cortex, whereas in the neocortex, they are mainly found in layers II and VI of the temporal and frontal lobes. Ultrastructurally, Pick bodies consist of random coiled and straight filaments.

Biochemical analysis, using a quantitative western blot approach with phosphorylation-dependent anti-tau antibodies has revealed that in all cases of Pick's disease studied, a major 55 and 64 kDa tau doublet is observed in the isocortex, in the limbic areas and in subcortical nuclei (Figure 5) (26). In addition, a very faint band is observed at 69 kDa (14). In the neocortex, all Brodmann areas of the frontal and temporal lobes are affected. The parietal cortex is frequently involved while the occipital cortex is generally spared. In subcortical structures, the tau doublet is found in the striatum, substantia nigra, locus coeruleus, and brainstem (26). The presence of the tau doublet correlated well with brain areas with Pick bodies (26). The 55 and 64 kDa doublet characteristic of Pick's disease is different from the tau-triplet in AD and tau-doublet in PSP and CBD (14, 85). Interestingly, Pick bodies and the tau doublet tau 55 and 64 are not labeled with immunological probes directed against the sequence encoded by exon 10 (85,115) indicating only 3R-tau isoforms aggregate into Pick bodies (Figure 5). Moreover, aggregated tau proteins in Pick's disease can not be detected by the monoclonal antibody 12E8 raised against the phosphorylated residue ser262. In contrast, this phosphorylation site is readily detected in other neurodegenerative disorders (28,102) (Figure 6). Since

it was shown that 3R-tau isoforms can be phosphorylated at Ser262, the lack of 12E8-immunoreactivity is likely to be related to either inhibition of a kinase in neurons that degenerate in Pick's disease or absence of these kinases within degenerating neurons (85). The present evidence suggests that only 3R-tau isoforms that are not phosphorylated at ser262 aggregate in Pick bodies (28, 85).

Frontal lobe degeneration non-Alzheimer non-Pick

Frontal lobe degeneration is a neurological disorder that has been not widely recognized until recently, despite the fact that it is the second most common presenile dementing disorder in Europe after AD. As in Pick's disease, it is associated with "frontal" pathology. Pick's disease is neuropathologically distinguished by the presence of Pick bodies, whereas frontal lobe degeneration has no specific neuropathologic hallmarks. Morphological changes include neuronal cell loss, spongiosis and gliosis mainly in the superficial cortical layers of the frontal and temporal cortex. No tau pathology is observed in this disorder (11, 27, 118).

FTDP-17

Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) has been related to mutations on the tau gene (65, 101, 118, 119). Tau mutations segregate with the pathology and are not found in the control subjects, suggesting their pathogenic role. Although clinical heterogeneity has been described between and within families with FTDP-17, the usual symptoms include behavioral changes, loss of frontal executive functions, language deficit and hyperorality. Parkinsonism and amyotrophy are described in some families. Neuropathologically, brains of FTDP-17 patients exhibit severe neuronal cell loss in frontal and temporal lobes and gliosis in both white and gray matter. One of the main histopathologic features is filamentous pathology affecting neuronal cells, or both neuronal and glial cells.

At present 20 mutations in the tau gene have been described among the different families with FTDP-17, including missense mutations in coding regions (K257T, I260V, G272V, N279K, L284L, P301L, P301S, S305N, V337M, G389R, R406W), amino acid deletions (ΔK280) and intronic mutations in the intronic region following exon 10 at position +3, +13, +14 and +16 (6, 16, 19, 30, 33, 34, 47, 50, 58, 64, 65, 67, 90, 94, 95, 101, 106, 108, 119, 126) (Table 2; Figure 7).

Mutations may be divided in two groups: 1) those affecting alternative splicing of exon 10 leading to changes in the ratio of tau mRNAs containing or lacking exon 10 and thus the proportion of 4R-and 3R-tau isoforms and 2) those modifying microtubule interactions. All intronic mutations disturb a putative stem-loop structure at the splicing site that stabilizes this region of the pre-mRNA and may decrease access of U1snRNP to this RNA region (50, 65, 119, 126). Without this stem loop, access of U1snRNP may be facilitated, which increases the formation of tau mRNAs containing exon 10 (33, 50, 126) (Figure 7). Furthermore, sequence analysis of this splicing region in different animals indicates that the lack of the stem-loop structure is associated with an increase in tau mRNAs containing exon 10 (50). All intronic mutations lead to an increase in tau mRNAs containing exon 10, and thus in 4R-tau isoforms. Interestingly, in those families, only abnormally phosphorylated 4R-tau isoforms aggregate into filaments and display a tau electrophoretic profile similar to that found in PSP and CBD (a major tau doublet at 64 and 69 kDa) (14, 40, 76, 118, 119, 129). Some missense mutations (N279K, ΔK280, L284L and S305N) also modify the splicing of exon 10 (33). For instance, the change in nucleotide for N279K and S305N mutations also creates an exon-splicing enhancer sequence (33). The silent mutation L284L increases the formation of tau mRNAs containing exon 10, presumably by destroying an exon splicing silencing element (33). Families exhibiting these three missense mutations display the same tau pathology (a tau doublet 64 and 69) as those with intronic mutations (Figures 5 and 7) (33, 64, 106).

The second group of tau mutations found in FTDP-17 includes mutations that alter the microtubule-binding properties of tau. Goedert and co-workers reported the effects of mutations G272V, P301L, V337M and R406W in an in vitro system of microtubule assembly. Mutated tau isoforms did not bind microtubules and induce microtubule disassembly as readily a normal tau (57). These data have been confirmed by additional laboratories (33, 64) and are discussed by Yen and co-workers in this symposium. When missense mutations are located in tau regions common to all isoforms, tau isoforms do not bind to microtubules as well as normal and they gradually aggregate into filaments. Their biochemical characterization shows a tau electrophoretic profile similar to that encountered in AD and is composed of a tau triplet (tau55, 64 and 69). Conversely, when missense mutations are located in exon 10 (P301L, P301S), only 4R-tau isoforms show poor binding to microtubules and subsequently aggregate into filaments. Their biochemical characterization shows a tau electrophoretic profile similar to that encountered in PSP and CBD

and is composed of a tau doublet (tau64 and 69) (Figures 5 and 7).

The ΔK280 mutation, which is located in exon 10, is a particularly interesting one. Despite being in a coding region, it may act similar to the splice site mutations by decreasing the formation of tau mRNAs containing exon 10 and thus, enhancing the formation of 3R tau isoforms. Interestingly, this tau missense mutation also affects tau binding. Thus, it should only affect 4R-tau isoforms. No data are currently available on the biochemistry of tau aggregates or the pathology in this family (108).

In summary, these findings suggest that reduced ability of tau to interact to microtubules may be upstream of hyperphosphorylation and aggregation. The tau mutations may also lead to an increase in free cytoplasmic tau (especially 4R-tau isoforms) that ultimately facilitates their aggregation into filaments (132). In this respect, it is interesting to note that over expression of tau was reported to block dynein-mediated axonal transport (35).

Conclusions

Despite the fact that many neurodegenerative disorders display specific electrophoretic tau profiles, it should be noted that there are overlapping patterns for some of them. The AD tau electrophoretic profile characterized by a major tau triplet tau 55, 64 and 69 and a minor variant at 74 kDa is also found in some forms of FTDP-17 (see above), amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam (12, 89), Down syndrome (39), Niemann-Pick type C disease (1), postencephalitic parkinsonism (15) and in the hippocampal formation in aging (29). The tau electrophoretic profile is identical for CBD and PSP even if their clinical features are different (116). Conversely, some disorders have unique electrophoretic tau profiles. For instance, PiD tau doublet has not been observed in any other disorder. Similarly, in myotonic dystrophy, tau pathology is mostly present in temporal areas and is characterized by an unique electrophoretic tau profile made of a major tau 55 variant (128). Whatever the electrophoretic tau profile, tau aggregation in association areas is always correlated to dementia (14, 15, 28, 29, 127, 129).

In conclusion, tau isoforms with 3R and 4R may be differentially expressed and their aggregation may lead to different biochemical signatures characterized by tau doublets and the tau triplet. Different processes may explain these observations. First, Goedert and co-workers (43) previously showed that neurons do not express 3R and 4R tau isoforms equally (for instance granule cells of the dentate gyrus express 3R-tau isoforms) and Delacourte and coworkers (28) clearly demonstrated that only 3R tau isoforms aggregate in Pick bodies in granule cells. Second, tau proteins are principally found in axons in normal neurons, but accumulate in somatodendritic neuronal compartments in neurodegenerative disorders. Since tau trafficking is phosphorylation-dependent (7, 10, 70, 105, 122), it suggests that abnormal phosphorylation of tau proteins may lead to aberrant cell trafficking and tau aggregation. Third, in some tauopathies, tau isoforms may be expressed in other cell types than neurons. For instance, tau aggregates are also found in glial cells (73). Finally, in hereditary disorders, differences in tau isoform expression are related to either mutations in tau (as in FTDP-17 or in tau polymorphisms (as in PSP). Most of tauopathies, however, including CBD, PiD, amyotrophic lateral sclerosis/parkinsonism dementia complex of Guam are not associated with tau mutations or polymorphisms (99, 101).

Altogether, these observations indicate that in many tauopathies, different processes including tau mutations or polymorphisms, aberrant cell trafficking and selective cell vulnerability act to determine specific patterns of neurodegeneration and corresponding tau biochemical profiles.

Acknowledgments

We gratefully acknowledge the support of the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, Immunotech (A Beckmann-Coulter Company), Rhône-Poulenc-Rorer, and Conseil Régional Nord Pas-de-Calais (Pôle Neurosciences). AD2 was developed through a collaboration between INSERM and UMR9921 CNRS-Sanofi Diagnostics Pasteur. We thank Drs Valérie Buée-Scherrer, Thierry Bussière, Michel Goedert, Patrick R. Hof, Mike Hutton, Jordi Pérez-Tur and Nicolas Sergeant for their expert comments.

References

1. Auer IA, Schmidt ML, Lee VM, Curry B, Suzuki K, Shin RW, Pentchev PG, Carstea ED, Trojanowski JQ (1995) Paired helical filament tau (PHFtau) in Niemann-Pick type C disease is similar to PHFtau in Alzheimer's disease. *Acta Neuropathol* 90: 547-551
2. Baker M, Litvan I, Houlden H, Adamson J, Dickson D, Perez-Tur J, Hardy J, Lynch T, Bigio E, Hutton M (1999) Association of an extended haplotype in the tau gene with progressive supranuclear palsy. *Hum Mol Genet* 8: 711-715.

3. Bennett P, Bonifati V, Bonuccelli U, Colosimo C, De Mari M, Fabbrini G, Marconi R, Meco G, Nicholl DJ, Stocchi F, Vanacore N, Vieregge P, Williams AC (1998) Direct genetic evidence for involvement of tau in progressive supranuclear palsy. European Study Group on Atypical Parkinsonism Consortium. *Neurology* 51: 982-985.
4. Bergeron C, Pollanen MS, Weyner L, Lang AE (1997) Cortical degeneration in progressive supranuclear palsy. A comparison with cortico-basal ganglionic degeneration. *J Neuropathol Exp Neurol* 56: 726-734
5. Biernat J, Gustke N, Drewes G, Mandelkow EM, Mandelkow E (1993) Phosphorylation of Ser(262) strongly reduces binding of tau proteins to microtubules - distinction between PHF-like immunoreactivity and microtubule binding. *Neuron* 11: 153-163
6. Bird TD, Nochlin D, Poorkaj P, Cherrier M, Kaye J, Payami ‘, Peskind E, Lampe TH, Nemens E, Boyer PJ, Schellenberg GD (1999) A clinical pathological comparison of three families with frontotemporal dementia and identical mutations in the tau gene (P301L). *Brain* 122: 741-756
7. Black MM, Slaughter T, Moshiach S, Obrocka M, Fischer I (1996) Tau is enriched on dynamic microtubules in the distal region of growing axons. *J Neurosci* 16: 3601-3619
8. Bramblett GT, Goedert M, Jakes R, Merrick SE, Trojanowski JQ, Lee VM (1993) Abnormal phosphorylation at Ser(396) in Alzheimer’s disease recapitulates development and contributes to reduced microtubule binding. *Neuron* 10: 1089-1099
9. Brion JP, Passareiro E, Nunez J, Flament-Durand J (1985) Immunological detection of tau protein in neurofibrillary tangles of Alzheimer’s disease. *Arch Biol* 95: 229-235
10. Brion JP, Octave JN, Couck AM (1994) Distribution of the phosphorylated microtubule-associated protein tau in developing cortical neurons. *Neuroscience* 63: 895-909
11. Brion S, Plas J, Jeanneau A (1991) Pick’s disease - a clinico-pathological point of view. *Rev Neurol* 147: 693-704
12. Buée-Scherrer V, Buée L, Hof PR, Leveugle B, Gilles C, Loerzel AJ, Perl DP, Delacourte A (1995) Neurofibrillary degeneration in amyotrophic lateral sclerosis/ parkinsonism-dementia complex of Guam - Immunochemical characterization of Tau proteins. *Am J Pathol* 146: 924-932
13. Buée-Scherrer V, Condamines O, Mourton-Gilles C, Jakes R, Goedert M, Pau B, Delacourte A (1996) AD2, a phosphorylation-dependent monoclonal antibody directed against Tau proteins found in Alzheimer’s disease. *Mol Brain Res* 39: 79-88
14. Buée-Scherrer V, Hof PR, Buée L, Leveugle B, Vermersch P, Perl DP, Olanow CW, Delacourte A (1996) Hyperphosphorylated Tau proteins differentiate corticobasal degeneration and Pick’s disease. *Acta Neuropathol* 91: 351-359
15. Buée-Scherrer V, Buée L, Leveugle B, Perl DP, Vermersch P, Hof PR, Delacourte A (1997) Pathological tau proteins in postencephalitic parkinsonism: comparison to Alzheimer’s disease and other neurodegenerative disorders. *Ann Neurol* 42: 356-359
16. Bugiani O, Murrell JR, Giaccone G, Hasegawa M, Ghigo G, Tabaton M, Morbin M, Primavera A, Carella F, Solaro C, Grisoli M, Savoiardo M, Spillantini MG, Tagliavini F, Goedert M, Ghetti B (1999) Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J Neuropathol Exp Neurol* 58: 595-605
17. Bussière T, Hof PR, Mailliot C, Brown C, Caillet-Boudin ML, Perl DP, Buée L, Delacourte A (1999) Phosphorylated serine422 on tau proteins is a pathological epitope found in several diseases with neurofibrillary degeneration. *Acta Neuropathol* 97: 221-230
18. Chin SSM, Goldman JE (1996) Glial inclusions in CNS degenerative diseases. *J Neuropathol Exp Neurol* 55: 499-508
19. Clark LN, Poorkaj P, Wszolek Z, Geschwind DH, Nasreddine ZS, Miller B, Li D, Payami ‘, Awert F, Markopoulou K, Andreadis A, D’Souza I, Lee VM, Reed L,Trojanowski JQ, Zhukareva V, Bird T, Schellenberg G, Wilhelmsen KC (1998) Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. *Proc Natl Acad Sci USA* 95: 13103-13107
20. Cleveland DW, Hwo SY, Kirschner MW (1977) Purification of tau, a microtubule-associated protein that induces assembly of microtubules from purified tubulin. *J Mol Biol* 116: 207-225
21. Cleveland DW, Hwo SY, Kirschner MW (1977) Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly. *J Mol Biol* 116: 227-247
22. Conrad C, Andreadis A, Trojanowski JQ, Dickson DW, Kang D, Chen X, Wiederholt W, Hansen L, Masliah E, Thal LJ, Katzman R, Xia ‘, Saitoh T (1997) Genetic evidence for the involvement of tau in progressive supranuclear palsy. *Ann Neurol* 41: 277-281
23. Conrad C, Amano N, Andreadis A, Xia ‘, Namekataf K, Oyama F, Ikeda K, Wakabayashi K, Takahashi ‘, Thal LJ, Katzman R, Shackelford DA, Matsushita M, Masliah E, Sawa A (1998) Differences in a dinucleotide repeat polymorphism in the tau gene between Caucasian and Japanese populations: implication for progressive supranuclear palsy. *Neurosci Lett* 250: 135-137
24. De Yebenes JG, Sarasa JL, Daniel SE, Lees AJ (1995) Familial progressive supranuclear palsy. Description of a pedigree and review of the litterature. *Brain* 118: 1095-1103
25. Delacourte A, Flament S, Dibe EM, Hublau P, Sablonnière B, Hémon B, Scherrer V, Défossez A (1990) Pathological proteins tau 64 and 69 are specifically expressed in the somatodendritic domain of the degenerating cortical neurons during Alzheimer’s disease, demonstration with a panel of antibodies against tau proteins. *Acta Neuropathol* 80: 111-117
26. Delacourte A, Robitaille Y, Sergeant N, Buée L, Hof PR, Wattez A, Laroche-Cholette A, Mathieu J, Chagnon P, Gauvreau D (1996) Specific pathological Tau protein variants characterize Pick’s disease. *J Neuropathol ExpNeurol* 55:159-168
27. Delacourte A, Buée L (1997) Normal and pathological tau proteins as factors for microtubule assembly. *Int Rev Cytol* 171: 167-224

28. Delacourte A, Sergeant N, Wattez A, Gauvreau D, Robitaille Y (1998) Vulnerable neuronal subsets in Alzheimer's and Pick's disease are distinguished by their tau isoform distribution and phosphorylation. *Ann Neurol* 43: 193-204

29. Delacourte A, David JP, Sergeant N, Buée L, Wattez A, Vermersch P, Ghozali F, Fallet-Bianco C, Pasquier F, Lebert F, Petit H, DiMenza C (1999) The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology* 52: 1158-1165

30. Delisle MB, Murrell JR, Richardson R, Trofatter JA, Rascol O, Soulages X, Mohr M, Calvas P, Ghetti B (1999) A mutation at codon 279 (N279K) in exon 10 of the tau gene causes a tauopathy with dementia and supranuclear palsy. *Acta Neuropathol* 98: 62-77

31. Dickson DW (1997) Neurodegenerative diseases with cytoskeletal pathology: a biochemical classification. *Ann Neurol* 42: 541-544

32. Drubin DG, Kirschner MW (1986) Tau protein function in living cells. *J Cell Biol* 103: 2739-2746

33. D'Souza I, Poorkaj P, Hong M, Nochlin D, Lee VMY, Bird TD, Schellenberg GD (1999) Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. *Proc Natl Acad Sci USA* 96: 5598-5603

34. Dumanchin C, Camuzat A, Campion D, Verpillat P, Hannequin D, Dubois B, Saugier-Veber P, Martin C, Penet C, Charbonnier F, Agid Y, Frebourg T, Brice A (1998) Segregation of a missense mutation in the microtubule-associated protein tau gene with familial frontotemporal dementia and parkinsonism. *Hum Mol Genet* 7: 1825-1829

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

36. Feany MB, Ksiezak-Reding ', Liu WK, Vincent I, Yen SHC, Dickson DW (1995) Epitope expression and hyperphosphorylation of tau protein in corticobasal degeneration: differentiation from progressive supranuclear palsy. *Acta Neuropathol* 90: 37-43

37. Feany MB, Mattiace LA, Dickson DW (1996) Neuropathologic overlap of progressive supranuclear palsy, Pick's disease and corticobasal degeneration. *J Neuropathol Exp Neurol* 55: 53-67

38. Flament S, Delacourte A (1989) Abnormal tau species are produced during Alzheimer's disease neurodegenerating process. *FEBS Lett* 247: 213-216

39. Flament S, Delacourte A, Mann DMA (1990) Phosphorylation of tau proteins: a major event during the process of neurofibrillary degeneration. Acomparative study between Alzheimer's disease and Down's syndrome. *Brain Res* 516: 15-19

40. Flament S, Delacourte A, Verny M, Hauw JJ, Javoy-Agid F (1991) Abnormal tau proteins in progressive supranuclear palsy. Similarities and differences with the neurofibrillary degeneration of the Alzheimer type. *Acta Neuropathol* 81: 591-596

41. Ginsberg SD, Crino PB, Lee VM, Eberwine JH, Trojanowski JQ (1997) Sequestration of RNA in Alzheimer's disease neurofibrillary tangles and senile plaques. *Ann Neurol* 41: 200-209

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

43. Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA (1989) Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing 4 tandem repeats - differential expression of tau protein messenger RNAs in human brain. *EMBO J* 8: 393-399

44. Goedert M, Spillantini MG, Cairns NJ, Crowther RA (1992) Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* 8: 159-168

45. Goedert M, Jakes R, Spillantini MG, Hasegawa M, Smith MJ, Crowther RA (1996) Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulfated glycosaminoglycans. *Nature* 383: 550-553

46. Goedert M, Spillantini MG, Davies SW (1998) Filamentous nerve cell inclusions in neurodegenerative diseases. *Curr Opin Neurobiol* 8: 619-632

47. Goedert M, Spillantini MG, Crowther RA, Chen SG, Parchi P, Tabaton M, Lanska DJ, Markesbery WR, Wilhelmsen KC, Dickson DW, Petersen RB, Gambetti P (1999) Tau gene mutation in familial progressive subcortical gliosis. *Nat Med* 5: 454-457

48. Goode BL, Denis PE, Panda D, Radeke MJ, Miller HP, Wilson L, Feinstein SC (1997) Functional interactions between the proline-rich and repeat regions of tau enhance microtubule binding and assembly. *Mol Biol Cell* 8: 353-365

49. Greenberg SG, Davies P, Schein JD, Binder LI (1992) Hydrofluoric acid-treated tau-PHF proteins display the same biochemical properties as normal tau. *J Biol Chem* 267: 564-569

50. Grover A, Houlden H, Baker M, Adamson J, Lewis J, Prihar G, Pickering-Brown S, Duff K, Hutton M (1999) 5'splice site mutations in tau associated with the inherited dementia FTDP-17 affect a stem loop structure that regulates alternative splicing of exon 10. *J Biol Chem* 274: 15134-15143

51. Grundke-Iqbal I, Iqbal K, Tung YC, Zaidi MS, Wisniewski HM, Binder LI (1986) Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA* 83: 4913-4917

52. Gu Y, Oyama F, Ihara Y (1996) Tau is widely expressed in rat tissues. *J Neurochem* 67:1235-1244

53. Hagestedt G, Lichtenberg B, Wille H, Mandelkow EM, Mandelkow M (1989) Tau protein becomes long and stiff upon phosphorylation: correlation between paracrystalline structure and degree of phosphorylation. *J Cell Biol* 109: 1643-1651

54. Hanger DP, Betts JC, Loviny TL, Blackstock WP, Anderton BH (1998) New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry. *J Neurochem* 71: 2465-2476

55. Hasegawa M, Morishima-Kawashima M, Takio K, Suzuki M, Titani K, Ihara Y (1992) Protein sequence and mass spectrometric analyses of tau in the Alzheimer's disease brain. *J Biol Chem* 267: 17047-17054

56. Hasegawa M, Jakes R, Crowther RA, Lee VMY, Ihara Y, Goedert M (1996) Characterization of mAb AP422, a novel phosphorylation-dependent monoclonal antibody against Tau protein. *FEBS Lett* 384: 25-30

57. Hasegawa M, Smith MJ, Goedert M (1998) Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly. *FEBS Lett* 437: 207-210

58. Hasegawa M, Smith MJ, Iijima M, Tabira T, Goedert M (1999) FTDP-17 mutations N279K and S305N in tau produce increased splicing of exon 10. *FEBS Lett* 443: 93-96

59. Hauw JJ, Verny M, Delaere P, Cervera P, He ', Duyckaerts C (1990) Constant neurofibrillary changes in the neocortex in progressive supranuclear palsy - basic differences with Alzheimer's disease and aging. *Neurosci Lett* 119: 182-186

60. Higgins JJ, Litvan I, Pho LT, Li W, Nee LE (1998) Progressive supranuclear gaze palsy is in linkage disequilibrium with the tau gene and not the alpha-synuclein gene. *Neurology* 50: 270-273

61. Hof PR, Delacourte A, Bouras C (1992) Distribution of cortical neurofibrillary tangles in progressive supranuclear palsy. A quantitative analysis of 6 cases. *Acta Neuropathol* 84: 45-51

62. Hof PR, Bouras C, Perl DP, Morrison JH (1994) Quantitative neuropathologic analysis of Pick's disease cases - cortical distribution of Pick bodies and coexistence with Alzheimer's disease. *Acta Neuropathol* 87: 115-124

63. Hoffmann R, Lee VMY, Leight S, Varga I, Otvos L (1997) Unique Alzheimer's disease paired helical filaments specific epitopes involve double phosphorylation at specific sites. *Biochemistry* 36: 8114-8124

64. Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L, Miller BI, Geschwind DH, Bird TD, McKeel D, Goate A, Morris JC, Wilhelmsen KC, Schellenberg GD, Trojanowski JQ, Lee VM (1998) Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. *Science* 282: 1914-1917

65. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowolny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski JQ, Basun ', Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd P, Hayward N, Kwok JBJ, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann DMA, Lynch T, Heutink P (1998) Coding and 5' splice site mutations in TAU associated with inherited dementia (FTDP-17). *Nature* 393: 702-705

66. Ihara Y, Nukina N, Miura R, Ogawara M (1986) Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease. *J Biochem* 99: 1807-1810

67. Iijima M, Tabira T, Poorkaj P, Schellenberg GD, Trojanowski JQ, Lee VMY, Schmidt ML, Takahashi K, Nakiba T, Matsumoto T, Yamashita ', Yoshioka S, Ishini H (1999) A distinct familial presenile dementia with a novel missense mutation in the tau gene. *NeuroReport* 10: 497-501

68. Johnson GVW, Hartigan JA (1998) Tau protein in normal and Alzheimer's disease brain: an update. *Alz Dis Rev* 3: 125-141

69. Kampers T, Friedhoff P, Biernat J, Mandelkow EM, Mandelkow E (1996) RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Lett* 399: 344-349

70. Kempf M, Clement A, Faissner A, Lee G, Brandt R (1996) Tau binds to the distal axon early in development of polarity in a microtubule- and microfilament-dependent manner. *J Neurosci* 16: 5583-5592

71. Kidd M (1963) Paired helical filaments in electron microscopy of Alzheimer's disease. *Nature* 197: 192-193

72. Komori T, Shibata N, Kobayashi M, Sasaki S, Iwata M (1998) Inducible nitric oxide synthase (iNOS)-like immunoreactivity in argyrophilic, tau-positive astrocytes in progressive supranuclear palsy. *Acta Neuropathol* 95: 338-344

73. Komori T (1999) Tau-positive glial inclusions in PSP, CBD and Pick's disease. *Brain Pathol* 9: this issue

74. Kondratick CM, Vandre DD (1996) Alzheimer's disease neurofibrillary tangles contain mitosis-specific phosphoepitopes. *J Neurochem* 67: 2405-2416

75. Kosik KS, Orecchio LD, Bakalis S, Neve RL (1989) Developmentally regulated expression of specific tau sequences. *Neuron* 2: 1389-1397

76. Ksiezak-Reding ', Morgan K, Mattiace LA, Davies P, Liu WK, Yen SH, Weidenheim K, Dickson DW (1994) Ultrastructure and biochemical composition of paired helical filaments in corticobasal degeneration. *Am J Pathol* 145: 1496-1508

77. Lee G, Cowan N, Kirschner M (1988) The primary structure and heterogeneity of Tau protein from mouse brain. *Science* 239: 285-289

78. Lee G, Neve RL, Kosik KS (1989) The microtubule binding domain of tau protein. *Neuron* 2: 1615-1624

79. Lee VMY, Balin BJ, Otvos L, Trojanowski JQ (1991) A68: a major subunit of paired helical filaments and derivatized forms of normal tau. *Science* 251: 675-678

80. Lindwall G, Cole RD (1984) Phosphorylation affects the ability of Tau protein to promote microtubule assembly. J *Biol Chem* 255: 5301-5305

81. Litvan I, Agid Y, Jankovic J, Goetz C, Brandel JP, Lai EC, Wenning G, Dolhaberriague L, Verny M, Chaudhuri R4, McKee A, Jellinger K, Bartko JJ, Mangone CA, Pearce RKB (1996) Accuracy of clinical criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome). *Neurology* 46: 922-930

82. Litvan I, Hauw JJ, Bartko JJ, Lantos PL, Daniel SE, Horoupian DS, McKee A, Dickson D, Bancher C, Tabaton M, Jellinger K, Anderson DW (1996) Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. *J Neuropathol Exp Neurol* 55: 97-105

83. Lovestone S, Reynolds CH (1997) The phosphorylation of tau: a critical stage in neurodevelopment and neurodegenerative processes. *Neuroscience* 78: 309-324

84. Lu PJ, Wulf G, Zhou XZ, Davies P, Lu KP (1999) The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* 399: 784-788

85. Mailliot C, Sergeant N, Bussière T, Caillet-Boudin ML, Delacourte A, Buée L (1998) Phosphorylation of specific sets of tau isoforms explains different neurodegeneration processes. *FEBS Lett* 433: 201-204

86. Mailliot C, Bussière T, Caillet-Boudin ML, Delacourte A, Buée L (1998). Alzheimer-specific epitope of AT100 in transfected cell lines with tau: Toward an efficient cell model of tau abnormal phosphorylation. *Neurosci Lett* 255: 13-16

87. Mandelkow EM, Mandelkow E (1998) Tau in Alzheimer's disease. *Trends Cell Biol* 8: 425-427

88. Matsuo ES, Shin RW, Billingsley ML, Vandevoorde A, Oconnor M, Trojanowski JQ, Lee VMY (1994) Biopsy-derived adult human brain Tau is phosphorylated at many of the same sites as Alzheimer's disease paired helical filament tau. *Neuron* 13: 989-1002

89. Mawal-Dewan M, Schmidt ML, Balin B, Perl DP, Lee VMY, Trojanowski JQ (1996) Identification of phosphorylation sites in PHF-Tau from patients with Guam amyotrophic lateral sclerosis/parkinsonism-dementia complex. *J Neuropathol Exp Neurol* 55: 1051-1059

90. Mirra SS, Murrell JR, Gearing M, Spillantini MG, Goedert M, Crowther RA, Levey AI, Jones R, Green J, Shoffner JM, Wainer BH, Schmidt ML, Trojanowski JQ, Ghetti B (1999) Tau pathology in a family with dementia and a P301L mutation in tau. *J Neuropathol Exp Neurol* 58: 335-345

91. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida ', Titani K, Ihara ' (1995) Proline-directed and non-proline-directed phosphorylation of PHF-tau. *J Biol Chem* 270: 823-829

92. Morris HR, Janssen JC, Bandmann O, Daniel SE, Rossor MN, Lees AJ, Wood NW (1999) The tau gene A0 polymorphism in progressive supranuclear palsy and related neurodegenerative diseases. *J Neurol Neurosurg Psychiatry* 66: 665-667.

93. Mulot SFC, Hughes K, Woodgett JR, Anderton BH, Hanger DP (1994) PHF-Tau from Alzheimer's brain comprises four species on SDS-PAGE which can be mimicked by an in vitro phosphorylation of human brain tau by glycogen synthase kinase-3β. *FEBS Lett* 349: 359-364

94. Murrell J, Zolo P, Spillantini MG, Crowther RA, Goedert M, Redi F, Pietrini P, Guazelli M, Ghetti B (1999) A mutation at codon 389 (G389R) in exon 13 of the tau gene causes frontotemporal dementia with numerous Pick Bodies. *Clin Neuropathol* 18: 145

95. Nasreddine ZS, Loginov M, Clark LN, Lamarche J, Miller BL, Lamontagne A, Zhukareva V, Lee VM, Wilhelmsen KC, Geschwind DH (1999) From genotype to phenotype: a clinical pathological, and biochemical investigation of frontotemporal dementia and parkinsonism (FTDP-17) caused by the P301L tau mutation. *Ann Neurol* 45: 704-715

96. Oliva R, Tolosa E, Ezquerra M, Molinuevo JL, Valldeoriola F, Burguera J, Calopa M, Villa M, Ballesta F (1998) Significant changes in the tau A0 and A3 alleles in progressive supranuclear palsy and improved genotyping by silver detection. *Arch Neurol* 55: 1122-1124

97. Paulus W, Selim M (1990) Corticonigral degeneration with neuronal achromasia and basal neurofibrillary tangles. *Acta Neuropathol* 81: 89-94

98. Perez M, Valpuesta JM, Medina M, Montejo de Garcini E, Avila J (1996) Polymerization of tau into filaments in the presence of heparin: the minimal sequence required for tau-tau interaction. *J Neurochem* 67: 1183-1190

99. Pérez-Tur J, Buée L, Morris ', Waring S, Onstead L, Wavrant-De Vrièze F, Crook R, Buée-Scherrer V, Hof PR, Perl DP, Petersen R, McGeer P, Delacourte A, Hutton M, Siddique T, Ahlskog EJ, Hardy J, Steele J (1999) Absence of mutations in the TAU gene in Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam. *Neurology* 52, in press

100. Pick A (1906) Über einen weiteren Symptomen Komplex im Rahmen der Dementia senilis, bedingt durch umschriebene stärkere Hirnatrophie (gemischte Apraxie). *Monatsschr Psychiatr Neurol* 19: 97-108

101. Poorkaj P, Bird TD, Wijernan E, Nemens E, Garruto RM, Anderson L, Andreadis A, Winderholt WC, Raskind M, Schellenberg GD (1998) Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol* 43: 815-825

102. Probst A, Tolnay M, Langui D, Goedert M, Spillantini MG (1996) Pick's disease: hyperphosphorylated tau protein segregates to the somatoaxonal compartment. *Acta Neuropathol* 92: 588-596

103. Rebeiz JJ, Kolodny EH, Richardson Jr EP (1967) Corticodentatonigral degeneration with neuronal achromasia: a progressive disorder of late adult life. *Trans Am Neurol Assoc* 92: 23-26

104. Rebeiz JJ, Kolodny EH, Richardson EP Jr (1968) Corticodentatonigral degeneration with neuronal achromasia. *Arch Neurol* 18: 20-33

105. Rebhan M, Vacun G, Rosner ' (1995) Complementary distribution of Tau proteins in different phosphorylation states within growing axons. *Neuroreport* 6: 429-432

106. Reed LA, Schmidt ML, Wszolek ZK, Balin BJ, Soontornniyomkij V, Lee VMY, Trojanowski JQ, Schelper RL (1998) The neuropathology of a chromosome 17-linked autosomal dominant parkinsonism and dementia ("Pallido-ponto-nigral degeneration"). *J Neuropathol Exp Neurol* 57: 588-601

107. Rinne JO, Lee MS, Thompson PD, Marsden CD (1994) Corticobasal degeneration. A clinical study of 36 cases. *Brain* 117: 1183-1196

108. Rizzu P, Van Swieten JC, Joose M, Hasegawa M, Stevens M, Tibben A, Niermeijer MF, Hillebrandt M, Ravid R, Oostra BA, Goedert M, Van Duijn CM, Heutink P (1999) High prevalence of mutations in the microtubule-associated protein tau in a population study of fronto-temporal dementia in the Netherlands. *Am J Hum Genet* 64: 414-421

109. Roder HM, Fracasso RP, Hoffman FJ, Witowsky JA, Davis G, Pellegrino CB (1997). Phosphorylation-dependent monoclonal Tau antibodies do not reliably report phosphorylation by extracellular signal-regulated kinase 2 at specific sites. *J Biol Chem* 272: 4509-4515

110. Rojo A, Pernaute RS, Fontan A, Ruiz PG, Honnorat J, Lynch T, Chin S, Gonzalo I, Rabano A, Martinez A, Daniel S, Pramsteller P, Morris H, Wood N, Lees A, Tabernero C, Nyggard T, Jackson AC, Hanson A, de Yebenes JG (1999) Clinical genetics of familial progressive supranuclear palsy. *Brain* 122: 1233-1245

111. Schmidt ML, Huang R, Martin JA, Henley J, Mawal-Dewan M, Hurtig HI, Lee VM, Trojanowski JQ (1996) Neurofibrillary tangles in progressive supranuclear palsy contain the same tau epitopes identified in Alzheimer's disease PHFtau. *J Neuropathol Exp Neurol* l55: 534-539

112. Schneider JA, Watts RL, Gearing M, Brewer RP, Mirra SS (1997) Corticobasal degeneration: neuropathologic and clinical heterogeneity. *Neurology* 48: 959-969

113. Sergeant N, Bussiere T, Vermersch P, Lejeune JP, Delacourte A (1995) Isoelectric point differentiates PHF-tau from biopsy-derived human brain Tau proteins. *Neuroreport* 6: 2217-2220

114. Sergeant N, David JP, Goedert M, Jakes R, Vermersch P, Buée L, Lefranc D, Wattez A, Delacourte A (1997) Two dimensional characterization of PHF-Tau from Alzheimer's disease: demonstration of an additional 74 kDa component and age-related biochemical modifications. *J Neurochem* 69: 834-844

115. Sergeant N, David JP, Lefranc D, Vermersch P, Wattez A, Delacourte A (1997) Different distribution of phosphorylated tau protein isoforms in Alzheimer's and Pick's diseases. *FEBS Lett* 412: 578-582

116. Sergeant N, Wattez A, Delacourte A (1999) Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively "exon 10" isoforms. *J Neurochem* 72: 1243-1249

117. Seubert P, Mawal-Dewan M, Barbour R, Jakes R, Goedert M, Johnson GVW, Litersky JM, Schenk D, Lieberburg I, Trojanowski JQ, Lee VMY (1995) Detection of phosphorylated Ser(262) in fetal tau, adult tau, and paired helical filament tau. *J Biol Chem* 270: 18917-18922

118. Spillantini MG, Bird TD, Ghetti B (1998) Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies. *Brain Pathol* 8: 387-402

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

120. Spillantini MG, Tolnay M, Love S, Goedert M (1999) Microtubule-associated protein tau, heparan sulphate and alpha-synuclein in several neurodegenerative diseases with dementia. *Acta Neuropathol* 97: 585-594.

121. Steele JC, Richardson JC, Olzewski J (1964) Progressive supranuclear palsy. A heterogeneous degeneration involving brain stem, basal ganglia and cerebellum with vertical gaze ans pseudobulbar palsy, nuchal dystonia and dementia. *Arch Neurol* 10: 333-359

122. Szendrei GI, Lee VMY, Otvos L (1993) Recognition of the minimal epitope of monoclonal antibody Tau-1 depends upon the presence of a phosphate group but not its location. *J Neurosci Res* 34: 243-249

123. Tellez-Nagel I, Wisniewski HM (1973) Ultrastructure of neurofibrillary tangles in Steele-Richardson-Olszewski syndrome. *Arch Neurol* 29: 324-327

124. Tomonaga M (1977) Ultrastructure of neurofibrillary tangles in progressive supranuclear palsy. *Acta Neuropathol* 37: 177-181

125. Trojanowski JQ, Lee VM (1995) Phosphorylation of paired helical filament tau in Alzheimer's disease neurofibrillary lesions: focusing on phosphatases. *FASEB J* 9: 1570-1576

126. Varani L, Hasegawa M, Spillantini MG, Smith MJ, Murrell JR, Ghetti B, Klug A, Goedert M, Varani G (1999) Structure of tau exon 10 splicing regulatory element RNA and destabilization by mutations of frontotemporal dementia and parkinsonism linked to chromosome 17. *Proc Natl Acad Sci USA* 6: 8229-8234

127. Vermersch P, Robitaille Y, Bernier L, Wattez A, Gauvreau D, Delacourte A (1994) Biochemical mapping of neurofibrillary degeneration in a case of progressive supranuclear palsy: Evidence for general cortical involvement. *Acta Neuropathol* 87: 572-577

128. Vermersch P, Sergeant N, Ruchoux MM, Hofmann-Radvanyi H, Petit H, Dewailly P, Delacourte A (1996) Specific Tau variants in the brain from patients with myotonic dystrophy. *Neurology* 47: 711-717

129. Vermersch P, Buée-Scherrer V, Buée L, David JP, Wattez A, Sergeant N, Hof PR, Agid Y, Perl DP, Olanow CW, Robitaille Y, Gauveau D, Petit H, Delacourte A (1997) Cortical mapping of pathological tau proteins in several neurodegenerative disorders. In: Hyman B, Duyckaerts C, Christen Y (eds) *Connections, cognition and Alzheimer's disease*, Springer-Verlag, Berlin pp. 41-52

130. Vincent I, Rosado M, Davies P (1996) Mitotic mechanisms in Alzheimer's disease? *J Cell Biol* 132: 413-425

131. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW (1975) A protein factor essential for microtubule assembly. *Proc Natl Acad Sci USA* 72: 1858-1862

132. Yen S (1999) Fibrillogenesis of tau: insights from tau mutations in FTDP-17. *Brain Pathol* 9: this issue

133. Zheng-Fischhöfer Q, Biernat J, Mandelkow EM, Illenberger S, Godemann R, Mandelkow E (1998) Sequential phosphorylation of tau by glycogen synthase kinase-3ß and protein kinase A at Thr212 and Ser214 generates the Alzheimer-specific epitope of antibody AT100 and requires a paired-helical-filament-like conformation. *Eur J Biochem* 252: 542-552