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Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: Identification as the microtubule-associated protein tau

(molecular pathology/neurodegenerative disease/neurofibrillary tangles)

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ABSTRACT **Screening of cDNA libraries prepared from the frontal cortex of an Alzheimer disease patient and from fetal human brain has led to isolation of the cDNA for a core protein of the paired helical filament of Alzheimer disease. The partial amino acid sequence of this core protein was used to design synthetic oligonucleotide probes. The cDNA encodes a protein of 352 amino acids that contains a characteristic amino acid repeat in its carboxyl-terminal half. This protein is highly homologous to the sequence of the mouse microtubule-associated protein tau and thus constitutes the human equivalent of mouse tau. RNA blot analysis indicates the presence of two major transcripts, 6 and 2 kilobases long, with a wide distribution in normal human brain. Tau protein mRNAs were found in normal amounts in the frontal cortex from patients with Alzheimer disease. The proof that at least part of tau protein forms a component of the paired helical filament core opens the way to understanding the mode of formation of paired helical filaments and thus, ultimately, the pathogenesis of Alzheimer disease.**

Abundant neurofibrillary tangles and senile plaques constitute the neuropathological characteristics of Alzheimer disease (1). The knowledge of their molecular nature represents an essential prerequisite toward understanding their mode of formation. This may in turn shed light on the etiology and pathogenesis of Alzheimer disease.

The amyloid beta protein has been identified as the main component of cerebrovascular and plaque amyloid (2, 3). By contrast, the molecular nature of the paired helical filament (PHF), the major tangle constituent (4), remains unknown. Immunological studies have identified several candidate proteins, such as the 160- and 200-kDa neurofilament subunits (5, 6) vimentin (7), microtubule-associated protein 2 (8, 9), microtubule-associated protein tau (10–13), β-amyloid (14), and ubiquitin (15, 16). Such studies suggest that these molecules may share epitopes with PHFs. However, they suffer from the inherent inability to distinguish between molecules that form an integral part of the PHF and material that is associated with or adheres to the filamentous structures. This difficulty is compounded by the fact that different proteins (e.g., neurofilament and microtubule-associated proteins) possess epitopes in common (17, 18).

This paper is the first in a series of three that describes a combined structural, biochemical, immunological, and molecular biological approach to identify the components of the PHF core. The core is defined as that part of the PHF structure remaining after Pronase treatment (19, 20). The strategy has been to develop methods for solubilizing the PHF or part of it, which is the nub of the biochemical problem, because of the well-known insolubility of the PHFs (21). This task is made all the more difficult because there is no functional or physiological assay for the protein(s) of the PHF. The only identification so far possible is the morphology of the PHFs at the electron microscope level, and here we would accept only experiments on isolated individual filaments, not on neurofibrillary tangles (in which other material might be occluded). One thus needs a label or marker for the PHF itself, which can at the same time be used to follow the steps of the biochemical purification. We have therefore tried to develop chemical and immunological labels specific for PHFs. The second paper (19) describes the purification of protein fragments monitored by a chemical label and by a monoclonal antibody raised against enriched Pronase-treated PHF preparations. The third paper (20) justifies the use of this antibody in the biochemical work by immunoelectron microscopy of filament cores that have been proteolytically stripped of some of the surface material but remain morphologically intact.

The present paper describes the molecular cloning of the cDNA for a PHF core protein and its identification as the microtubule-associated protein tau.‡ Although there have been numerous immunological indications for the presence of tau protein-like immunoreactivity in neurofibrillary tangles or PHFs (10–13), we now have direct proof that at least part of tau forms a component of the core of the PHF.

MATERIALS AND METHODS

Construction of cDNA Libraries and Screening. RNA was isolated, by a modification of the guanidinium isothiocyanate/hot phenol technique (22), from the brain of a 15-week-old human fetus and from the frontal cortex of a 65-year-old patient who had died with a histologically confirmed diagnosis of Alzheimer disease; the cortical tissue was obtained 3 hr after death. Poly(A)$^+$ RNAs were enriched for by oligo(dT)-cellulose affinity chromatography (23). First-strand cDNA synthesis was carried out by using murine reverse transcriptase in the presence of actinomycin D (40 μg/ml) with oligo(dT) as a primer. Double-stranded cDNA was generated by a modification of the procedure described by Gubler and Hoffman (24), using RNase H, DNA polymerase I, and *Escherichia coli* DNA ligase. After treatment with S1 nuclease and *Eco*RI methylase, the size-selected double-stranded cDNA was cloned into the imm^{434} *Eco*RI insertion vector λgt10 (25) with *Eco*RI linkers. Fetal poly(A)$^+$ RNA (10 μg) yielded a library of 4×10^6 clones and 10 μg of frontal cortical poly(A)$^+$ RNA resulted in 6.2×10^6 clones.

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Abbreviation: PHF, paired helical filament.

‡The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Intelligenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03778).

Replica filters were screened with two mixed synthetic oligonucleotide probes, [5′ GG$\left(\begin{smallmatrix}T\\C\end{smallmatrix}\right)TT\left(\begin{smallmatrix}A\\G\end{smallmatrix}\right)TA\left(\begin{smallmatrix}T\\C\end{smallmatrix}\right)AC\left(\begin{smallmatrix}A\\G\\T\end{smallmatrix}\right)AT\left(\begin{smallmatrix}T\\C\end{smallmatrix}\right)$TG 3′] and [5′ GG$\left(\begin{smallmatrix}T\\C\end{smallmatrix}\right)TT\left(\begin{smallmatrix}A\\G\end{smallmatrix}\right)TA\left(\begin{smallmatrix}A\\G\end{smallmatrix}\right)AC\left(\begin{smallmatrix}A\\G\\T\end{smallmatrix}\right)AT\left(\begin{smallmatrix}T\\C\end{smallmatrix}\right)$TG 3′], derived from the amino acid sequence Gln-Ile-Val-Tyr-Lys-Pro of part of a PHF core protein (see ref. 19 for purification, identification, and amino acid sequence determination). The probes were labeled with [γ-^{32}P]ATP by using T4 polynucleotide kinase. Hybridization-positive clones were plaque-purified and the melting profile of the hybrids was determined by using 3 M tetramethylammonium chloride/50 mM Tris·HCl, pH 8.0/2 mM EDTA, containing 1 mg of NaDodSO$_4$ per ml (26). In some experiments, replica filters were screened with restriction fragments labeled by the random-primer technique (27).

DNA Sequencing. Sequencing of both strands of DNA was performed by a modification (28) of the dideoxy chain-termination method (29) with overlapping restriction fragments subcloned into M13mp18 and M13mp19 and synthetic oligonucleotides as primers.

RNA Blot Analysis. A 1.1-kilobase (kb) *Eco*RI/*Nhe* I fragment from the insert of the cDNA clone λPHF7 was subcloned into M13mp18 and single-stranded DNA probes were prepared as described (30), resulting in a labeled 0.45-kb *Pst* I/*Nhe* I fragment from the coding region.

Human tissues were obtained <6 hr after death and were stored at −70°C until use. Eight different brain regions and three peripheral tissues were dissected from a 72-year-old patient who had died with no neurological or psychiatric disorders. The frontal cortex was used from the 72-year-old patient and from three others who had also died without neurological or psychiatric disease (ages 68, 74, and 79 years) and from four patients who had died with a histologically confirmed diagnosis of Alzheimer disease (ages 65, 78, 80, and 83 years). RNA extractions, hybridizations, and washings were performed as described (30). After dehybridization, the blots were rehybridized with a mouse β-actin cDNA clone (30) labeled by the random-primer technique (27).

RESULTS

Isolation of cDNA Clones. A portion of the cDNA library prepared from the frontal cortex of a patient who had died with Alzheimer disease was screened by using the two 17-mer oligonucleotides described above. A single hybridization-positive clone was obtained from 650,000 clones; the melting temperature of the hybrids was 56°C, suggesting a perfect match of the cDNA clone with one of the oligonucleotide probes. This was confirmed by sequencing a 160-base-pair hybridization-positive *Hae* III/*Alu* I fragment of this clone. It was found to encode the peptide sequence, only part of which had been used for designing the oligonucleotide probes. Screening of ≈50,000 clones from the fetal brain cDNA library with the *Hae* III/*Alu* I fragment resulted in 34 additional positives, several of which were plaque-purified. Two of these clones (λPHF5 and λPHF7) were further characterized. The exact insert size of λPHF5 is unknown, as it could not be excised with *Eco*RI. However, a partial restriction map indicates a length of 2.9 kb. The cDNA insert of λPHF7 is 2.8 kb long.

Nucleotide Sequence of cDNA Clones and Deduced Amino Acid Sequence: Identity with Tau Protein. A partial nucleotide sequence determined from clones PHF5 and PHF7, which encompasses the complete coding region, is depicted in Fig. 1. It consists of 37 nucleotides of 5′ untranslated region, an open reading frame of 1056 nucleotides, an in-frame stop codon, and 12 nucleotides of 3′ untranslated sequence. The translation initiation site was assigned to the methionine codon numbered as nucleotides 1–3, as it is the first ATG downstream of an in-frame stop codon (nucleotides −9 to −7). The open reading frame encodes a protein of 352 amino acids, whose most striking feature is a stretch of 31 or 32 amino acids that is repeated three times (amino acids 198–228, 229–260, and 261–292) (Fig. 2). The protein sequence contains four putative calcium/calmodulin-dependent protein kinase (31) phosphorylation sites (residues 71, 156, 263, and 320), five putative protein kinase C (31) phosphorylation sites (residues 73, 91, 152, 183, and 199), one

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                                                                    -37 CCGCCTCTGTCGACTATCAGGTGAACTTTGAACCAGG

  1 Met Ala Glu Pro Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu Gly Asp Arg Lys Asp Gln Gly Gly Tyr Thr
  1 ATG GCT GAG CCC CGC CAG GAG TTC GAA GTG ATG GAA GAT CAC GCT GGG ACG TAC GGG TTG GGG GAC AGG AAA GAT CAG GGG GGC TAC ACC

 31 Met His Gln Asp Gln Glu Gly Asp Thr Asp Ala Gly Leu Lys Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro Ser Leu Glu Asp Glu Ala
 91 ATG CAC CAA GAC CAA GAG GGT GAC ACG GAC GCT GGC CTG AAA GCT GAA GAA GCA GGC ATT GGA GAC ACC CCC AGC CTG GAA GAC GAA GCT

 61 Ala Gly His Val Thr Gln Ala Arg Met Val Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys Ala Lys Gly Ala Asp Gly Lys
181 GCT GGT CAC GTG ACC CAA GCT CGC ATG GTC AGT AAA AGC AAA GAC GGG ACT GGA AGC GAT GAC AAA AAA GCC AAG GGG GCT GAT GGT AAA

 91 Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro Pro Ala
271 ACG AAG ATC GCC ACA CCG CGG GGA GCA GCC CCT CCA GGC CAG AAG GGC CAG GCC AAC GCC ACC AGG ATT CCA GCA AAA ACC CCG CCC GCT

121 Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser
361 CCA AAG ACA CCA CCC AGC TCT GGT GAA CCT CCA AAA TCA GGG GAT CGC AGC GGC TAC AGC AGC CCC GGC TCC CCA GGC ACT CCC GGC AGC

151 Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser
451 CGC TCC CGC ACC CCG TCC CTT CCA ACC CCA CCC ACC CGG GAG CCC AAG AAG GTG GCA GTG GTC CGT ACT CCA CCC AAG TCG CCG TCT TCC

181 Ala Lys Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His
541 GCC AAG AGC CGC CTG CAG ACA GCC CCC GTG CCC ATG CCA GAC CTG AAG AAT GTC AAG TCC AAG ATC GGC TCC ACT GAG AAC CTG AAG CAC

211 Gln Pro Gly Gly Gly Lys Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His
631 CAG CCG GGA GGC GGG AAG GTG CAA ATA GTC TAC AAA CCA GTT GAC CTG AGC AAG GTG ACC TCC AAG TGT GGC TCA TTA GGC AAC ATC CAT

241 His Lys Pro Gly Gly Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser Lys Ile Gly Ser Leu Asp Asn
721 CAT AAA CCA GGA GGT GGC CAG GTG GAA GTA AAA TCT GAG AAG CTT GAC TTC AAG GAC AGA GTC CAG TCG AAG ATT GGG TCC CTG GAC AAT

271 Ile Thr His Val Pro Gly Gly Gly Asn Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly
811 ATC ACC CAC GTC CCT GGC GGA GGA AAT AAA AAG ATT GAA ACC CAC AAG CTG ACC TTC CGC GAG AAC GCC AAA GCC AAG ACA GAC CAC GGG

301 Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser Ile Asp Met
901 GCG GAG ATC GTG TAC AAG TCG CCA GTG GTG TCT GGG GAC ACG TCT CCA CGG CAT CTC AGC AAT GTC TCC TCC ACC GGC AGC ATC GAC ATG

331 Val Asp Ser Pro Gln Leu Ala Thr Leu Ala Asp Glu Val Ser Ala Ser Leu Ala Lys Gln Gly Leu ***
991 GTA GAC TCG CCC CAG CTC GCC ACG CTA GCT GAC GAG GTG TCT GCC TCC CTG GCC AAG CAG GGT TTG TGA TCAGGCCCCTGG
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FIG. 1. Nucleotide and predicted amino acid sequences of one form of human tau protein, as deduced from clones PHF5 and PHF7. Nucleotides are numbered in the 5′ → 3′ direction, starting with the first nucleotide of the initiating methionine; the nucleotides 5′ to residue 1 are indicated by negative numbers.

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(1)  Val Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly Gly Lys Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys
(2)  Val Thr Ser Lys Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg
(3)  Val Gln Ser Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn
      *     *  *  +  *  *  +     *  #     *     *  *  *  *     +     #           +  #  +  #
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FIG. 2. Three tandem repeats (31 or 32 amino acids long) identified in the human brain tau protein sequence, corresponding to amino acids 198–228, 229–260, and 261–292. Similarity is indicated as follows: *, all three residues are identical; #, two residues are identical and the third is a conservative replacement; +, two residues are identical and the third is different.

potential tyrosine kinase (31) phosphorylation site at residue 139, and the sequence presumed to be phosphorylated in the middle neurofilament subunit (32) at residues 306–309.

An RNA blot (data not shown), with the 160-base-pair *Hae* III/*Alu* I fragment used as a probe, indicated the presence of a 6- and a 2-kb transcript in human frontal cortex, with no detectable signal in heart or kidney. After dehybridization, the same blot was rehybridized with the 1.9-kb *Pst* I insert from the mouse tau clone pTA2 (33), which was a gift from M. Kirschner (University of California, San Francisco). The fact that the patterns of hybridization and tissue distribution were very similar to the one obtained with the *Hae* III/*Alu* I fragment strongly suggested that we had cloned the cDNA for human tau. This has now been firmly established with the recent publication of the cloning and sequencing of cDNAs for mouse tau (34); our sequence corresponds to the shorter of the two mouse forms.

Distribution of Tau Protein mRNA. The tissue distribution of tau protein mRNA was investigated by using poly(A)$^+$ RNA prepared from frontal cortex, hippocampus, basal forebrain, striatum, thalamus, midbrain, amygdala, cerebellum, heart, kidney, and adrenal gland from a patient who had died with no neurological or psychiatric disorders (Fig. 3). Two major transcripts of 6 and 2 kb and several minor ones were observed by RNA blotting, and substantial levels of tau protein mRNA were present in all brain regions, with no detectable signal in the peripheral tissues investigated (Fig. 3*a*). Only small regional differences were observed when the results were normalized with a mouse β-actin cDNA clone (Fig. 3*b*). No hybridization was observed with poly(A)$^-$ RNA.

The levels of tau protein mRNA were investigated in the frontal cortex from four control patients and from four patients who had died with a histologically confirmed diagnosis of Alzheimer disease (Fig. 4). The results were normalized by using the mouse β-actin clone (Fig. 4*b*). The same major 6- and 2-kb transcript bands were observed in both groups, and no overall change in tau protein mRNA levels was found in Alzheimer disease (Fig. 4*a*).

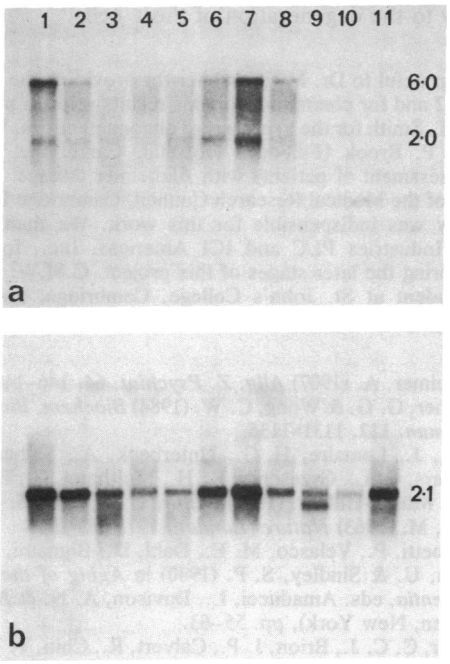

FIG. 3. RNA blot analysis of poly(A)$^+$ RNA from human control tissues, with ^{32}P-labeled human tau DNA (*a*) or mouse β-actin DNA (*b*) used as probe. Each lane contained 5 μg of poly(A)$^+$ RNA. Lanes: 1, frontal cortex; 2, hippocampus; 3, basal forebrain; 4, striatum; 5, thalamus; 6, midbrain; 7, amygdala; 8, cerebellum; 9, heart; 10, kidney; 11, adrenal gland. The blot was rehybridized with the actin probe after dehybridization of the tau probe. The lower band in lane 9 corresponds to cardiac actin. The 0.24- to 9.5-kb RNA ladder (Bethesda Research Laboratories) served as a size marker. The autoradiographic exposure times were 14 hr for *a* and 3 hr for *b*.

DISCUSSION

The present results provide the complete amino acid sequence of one form of human tau and, taken in conjunction with the accompanying papers (19, 20), directly demonstrate that at least part of the microtubule-associated protein tau constitutes a component of the PHF core in Alzheimer disease. The accompanying papers describe the isolation and partial amino acid sequencing of protein fragments that react with a monoclonal antibody (19) that also decorates the Pronase-stripped PHF core (20). These protein fragments thus form an integral part of the PHF core. Previously, tau protein had been shown to be one of several proteins that

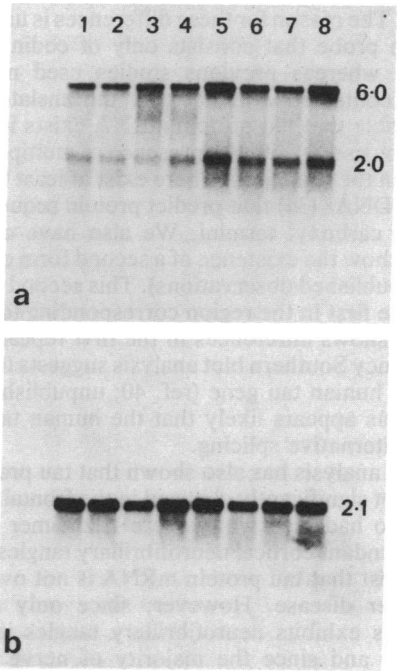

FIG. 4. RNA blot analysis of poly(A)$^+$ RNA from frontal cortex of control patients and of patients who had died with Alzheimer disease, with ^{32}P-labeled human tau DNA (*a*) or mouse β-actin DNA (*b*) used as a probe. Each lane contained 5 μg of poly(A)$^+$ RNA. Lanes: 1–4, frontal cortex from control patients; 5–8, frontal cortex from Alzheimer disease patients. Other details are the same as for Fig. 3.

share epitopes with neurofibrillary tangles (5–16). However, these earlier studies could not distinguish between epitopes of PHF core protein and those of material that adheres to or is loosely associated with PHFs.

Comparison of human and mouse (34) tau indicates that tau is a highly conserved protein. The two sequences are identical from human residue 200 to the carboxyl terminus of the protein, but in the amino-terminal part there are 25 amino acid changes and a small insertion and deletion. The most notable difference is the intercalation of a stretch of 11 amino acids starting at residue 16 of human tau. Both mouse and human tau contain a conserved 31- or 32-amino acid stretch that is repeated three times, each containing a distinctive Pro-Gly-Gly-Gly motif. As previously suggested (34), these repeats might represent the tubulin-binding sites of tau, and certainly the identity of sequences from these repeats to the carboxyl terminus suggests a conserved structural interface. Prediction of secondary structure (35) suggests the likely presence of reverse turns at each Pro-Gly-Gly-Gly and of α-helices in the carboxyl-terminal parts of the second and third repeats. It is notable that proline, glycine, and charged amino acids account for almost half the sequence, suggestive of an extended structure (36).

The results of the RNA blotting experiments indicate that tau protein mRNA is widely distributed throughout the human brain, with very little variation between different brain regions. No tau protein mRNA could be detected in the peripheral tissues investigated, suggesting that tau may be a nervous system-specific microtubule-associated protein. This is supported by its abundance in brain and its absence in fibroblasts (37, 38). Two major transcripts, 6 and 2 kb long, were detected in the different human brain regions. Additional weaker bands observed did not result from general degradation, as minor bands were not observed with the actin probe. RNA blots have previously shown a single transcript of 6 kb for tau protein mRNA in mouse (33), rat (39), and human (40) brain. However, *in vitro* translation of size-fractionated RNA gave a size of 2.2 kb for rat brain tau mRNA (41). The reason for these differences is unknown. We have used a probe that consists only of coding region for human tau, whereas previous studies used much longer probes that contained stretches of 3′ untranslated region.

It is probable that tau protein mRNA exists in more than one form, consistent with the presence of multiple species of tau protein in the brain (37). There exist at least two types of mouse tau cDNAs (34) that predict protein sequences differing at their carboxyl termini. We also have other cDNA clones that show the existence of a second form of human tau protein (unpublished observations). This second form is very similar to the first in the region corresponding to the protein repeats but shows differences in the first repeat. However, high-stringency Southern blot analysis suggests the existence of only one human tau gene (ref. 40; unpublished observations). It thus appears likely that the human tau mRNA is subject to alternative splicing.

RNA blot analysis has also shown that tau protein mRNA levels are not significantly changed in the frontal cortex from patients who had died with severe Alzheimer disease and thus had abundant cortical neurofibrillary tangles. This result would suggest that tau protein mRNA is not overexpressed in Alzheimer disease. However, since only a subset of cortical cells exhibits neurofibrillary tangles in Alzheimer disease (42) and since the majority of nerve cells in the cerebral cortex is likely to express tau protein mRNA that can exist in more than one form, a detailed analysis of tau protein mRNAs in Alzheimer disease is now mandatory. Perhaps certain tau protein mRNAs are specifically associated with the cell types that develop neurofibrillary tangles in Alzheimer disease and this confers selective vulnerability on these cells. It should be possible to resolve this issue by characterizing the different tau protein mRNAs, developing probes specific for each form, and investigating their expression by *in situ* hybridization.

It is not known why at least part of the soluble tau protein is present in an insoluble form in the PHF core in Alzheimer disease and how this relates to the etiology of the disease. Some studies have shown that tau protein is phosphorylated in neuronal cell bodies in Alzheimer disease but not in normal human brain (10–12, 43). However, at least one study shows tau to be phosphorylated in neuronal cell bodies and dendrites in normal rat brain (44). Purified tau protein can be phosphorylated by a calcium/calmodulin-dependent kinase (45) and by protein kinase C (46, 47). There are in the sequence described above several potential phosphorylation sites for these protein kinases, and in addition, one putative tyrosine kinase phosphorylation site. Recent experiments have indicated that the staining of neurofibrillary tangles with at least some antisera raised against the 160- and 200-kDa neurofilament subunits is due to cross-reactivity with phosphorylated tau (17, 18). The presence in human tau of the sequence Lys-Ser-Pro-Val, which constitutes the postulated phosphorylation site of the human 160-kDa neurofilament subunit (32), could explain this observation.

The genetic defect responsible for a rare autosomal dominant form of Alzheimer disease is located on the long arm of chromosome 21 (48). The human tau gene maps to chromosome 17 (40), so an inherited defect in the tau protein gene is not the cause of familial Alzheimer disease. One physiological function of tau protein is to promote microtubule assembly (35, 49). The fact that a large proportion of the total cellular pool of tau protein is immobilized in the nerve cells that contain neurofibrillary tangles implies that sufficient amounts of tau protein may not be available for maintaining microtubules in their active state. This would eventually result in severe impairment of axonal transport, leading ultimately to the degeneration of these cells.

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