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From genetics to pathology: tau and α-synuclein assemblies in neurodegenerative diseases

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The most common degenerative diseases of the human brain are characterized by the presence of abnormal filamentous inclusions in affected nerve cells and glial cells. These diseases can be grouped into two classes, based on the identity of the major proteinaceous components of the filamentous assemblies. The filaments are made of either the microtubule-associated protein tau or the protein α-synuclein. Importantly, the discovery of mutations in the tau gene in familial forms of frontotemporal dementia and of mutations in the α-synuclein gene in familial forms of Parkinson's disease has established that dysfunction of tau protein and α-synuclein can cause neurodegeneration.

Keywords: tau protein; Alzheimer's disease; Pick's disease; α-synuclein; Lewy body diseases; multiple system atrophy

1. INTRODUCTION

Alzheimer's disease and Parkinson's disease are major neurodegenerative diseases, with Alzheimer's disease being the most common dementing condition and Parkinson's disease the most common movement disorder. Both diseases are characterized by the degeneration of selected populations of nerve cells that develop abundant intracytoplasmic filamentous inclusions prior to degeneration. Similar inclusions are also found in a number of other, less common, neurodegenerative diseases. Recent work has shown that these inclusions are made of either the microtubule-associated protein tau or the protein α-synuclein (Goedert 1999), providing a simple and general underlying theme to the study of the vast majority of cases of late-onset neurodegenerative disease.

It is well established that the number of intracellular filamentous inclusions and their regional distribution correlate with the clinical picture in at least some neurodegenerative diseases. However, until recently, there was no evidence for a causal link. This has changed dramatically with the discovery of tau gene mutations in familial forms of frontotemporal dementia (Poorkaj *et al.* 1998; Hutton *et al.* 1998; Spillantini *et al.* 1998*a*) and of α-synuclein gene mutations in rare cases of familial Parkinson's disease (Polymeropoulos *et al.* 1997; Krüger *et al.* 1998). These discoveries have shown that the pathway leading to the formation of tau or α-synuclein filaments is central to the aetiology and the pathogenesis of these familial forms of disease. The same may be true of the much more common forms of sporadic disease. Here we review what is known about tau and α-synuclein assemblies and the mechanisms underlying their formation.

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2. TAU PROTEIN

Tau is a microtubule-associated protein the physiological functions of which are to promote microtubule assembly and to stabilize microtubules (Hirokawa 1994). In adult human brain, six tau isoforms ranging from 352 to 441 amino acids in length are produced from a single gene by alternative mRNA splicing (figure 1*a*) (Goedert *et al.* 1988, 1989*a*,*b*; Goedert & Jakes 1990; Andreadis *et al.* 1992). They differ from one another by the presence or absence of 29- or 58-amino-acid inserts located in the N-terminal half and a 31-amino-acid repeat located in the C-terminal half. Inclusion of the latter, which is encoded by exon 10 of the tau gene, gives rise to the three tau isoforms with four repeats each. The other three isoforms have three repeats each. In normal cerebral cortex, similar levels of three-repeat and four-repeat tau isoforms are found. Tau isoforms with four repeats are better at promoting microtubule assembly than isoforms with three repeats (Goedert & Jakes 1990). The repeats and some adjoining sequences constitute the microtubule-binding domains of tau, with the functions of the N-terminal region remaining uncertain (Gustke *et al.* 1994; Trinczek *et al.* 1995). Tau expression is developmentally regulated, in that only the isoform with three repeats and no N-terminal inserts is present in foetal human brain (Goedert *et al.* 1989*a*,*b*). Tau protein mRNA is expressed predominantly in nerve cells, with lower levels in some glial cells. Within nerve cells, tau protein is found mainly in axons (Binder *et al.* 1985). Inactivation of the tau gene by homologous recombination has been shown to lead to

a decrease in the number of microtubules in small-calibre axons, signs of muscle weakness and impaired contextual fear conditioning (Harada *et al.* 1994; Ikegami *et al.* 2000). The tau sequences from several vertebrate species are very similar, especially in the microtubule-binding repeat region. Proteins with tau-like repeats have been identified in *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* (Irminger-Finger *et al.* 1996; Goedert *et al.* 1996*a*; Rubin *et al.* 2000). Tau is believed to be a natively unfolded protein with little ordered secondary structure (Cleveland *et al.* 1977; Schweers *et al.* 1994). It is resistant to heat and to dilute acid, without losing its ability to promote microtubule assembly. Under electron microscopy, it looks rod-like, reflecting a largely extended conformation. When binding to microtubules, tau loses some of its flexibility and probably becomes structured (Woody *et al.* 1983). The nature of this conformational change is not known.

Tau is a phosphoprotein and phosphorylation is also developmentally regulated. Thus, the shortest tau isoform is phosphorylated more during development than any of the six tau isoforms in adult brain (Kanemura *et al.* 1992; Goedert *et al.* 1993). Phosphorylation sites in tau can be classified as either proline-directed or non-proline-directed sites (Morishima-Kawashima *et al.* 1995). A number of protein kinases have been implicated in the phosphorylation of tau, largely based on studies of phosphorylation *in vitro*. Protein phosphatase 2A is the major protein phosphatase activity in brain able to dephosphorylate tau phosphorylated by a number of protein kinases (Goedert *et al.* 1992*a*). It has also been shown to bind directly to the microtubule-binding region of tau (Sontag *et al.* 1999).

Relatively little is known about which protein kinases phosphorylate tau in the brain. To determine this requires specific protein kinase inhibitors or the inactivation of individual protein kinase genes. The use of lithium chloride as a specific inhibitor of glycogen synthase kinase-3 (GSK3) has provided strong evidence that this protein kinase is involved in the phosphorylation of tau in normal brain (Munoz-Montano *et al.* 1997; Hong *et al.* 1997). Phosphorylation negatively regulates the ability of tau to interact with microtubules, suggesting that it may induce a conformational change in tau (Bramblett *et al.* 1993; Yoshida & Ihara 1993). Interestingly, the organic osmolyte trimethylamine N-oxide has been shown to overcome the functional deficit of tau phosphorylated by cAMP-dependent protein kinase or GSK3 (Tseng *et al.* 1999). A similar effect has been observed upon binding of the *cis–trans* peptidyl prolyl isomerase Pin1 to phosphorylated tau (Lu *et al.* 1999). Pin1 has been shown to bind to a single phosphothreonine residue (T231) in tau.

3. DISEASES WITH TAU PATHOLOGY

(a) *Alzheimer's disease*

Unlike most neurodegenerative diseases, Alzheimer's disease is defined by the presence of two types of filamentous deposits, one extracellular and one intracellular. The extracellular deposits contain Aβ amyloid as their major component, a 40–42 amino-acid cleavage product of the much larger amyloid precursor protein (APP) (Glenner & Wong 1984; Masters *et al.* 1985; Kang *et al.* 1987). Hyperphosphorylated tau protein forms the major component of the intracellular neurofibrillary lesions that are seen as neurofibrillary tangles, neuropil threads and dystrophic neurites. Ultrastructurally, they consist of paired helical filaments (PHFs) and structurally related straight filaments (SFs), the characteristic morphologies of which were first visualized in plastic-embedded sections (Kidd 1963). The relationship between Aβ deposits and neurofibrillary lesions is at present unknown.

Clumps of PHFs can be isolated from Alzheimer's disease brain as tangle fragments (Wischik *et al.* 1985), but dispersed filaments are best prepared as a sarkosyl-insoluble pellet (Greenberg & Davies 1990; Lee *et al.* 1991). Electron micrographs of negatively stained isolated filaments show images in which the width of the filament appears to vary between *ca.* 8 nm and 20 nm, with a spacing between crossovers of *ca.* 80 nm. Rare examples of half-PHFs, split longitudinally along the axis, establish the double-stranded nature of the packing (Wischik *et al.* 1985). Fragments of filaments show no discernible preferred lengths but show sharp transverse breaks and an absence of fraying, indicating that each subunit in the packing extends a relatively small distance parallel to the axis. The computed cross-section of the filament shows two C-shaped structural units, arranged base to base, giving overall an elongated profile (Crowther & Wischik 1985). As the two strands of subunits twist around one another they create a double-helical structure, the image of which shows the characteristic alternating wide and narrow regions. SFs, a minor species of abnormal filament, also consist of two strands but arranged such that the C-shaped subunits contact back-to-back, giving the SFs a more isometric cross-section (Crowther 1991*a*). Images of SFs consequently do not show the marked alternation in width exhibited by PHFs. The secondary structure characteristics of tau filaments from Alzheimer's disease brain are not clear. One study using tangle fragments reported a cross-β conformation characteristic of amyloids (Kirschner *et al.* 1986), whereas a subsequent study on dispersed tau filaments failed to find detectable secondary structure by X-ray diffraction or spectroscopy (Schweers *et al.* 1994).

PHFs and SFs consist of hyperphosphorylated tau protein and dispersed filaments are strongly decorated by antibodies against the N- and C-terminal regions of tau, indicating that they contain the whole of tau (Goedert 1999). All six tau isoforms are present in dispersed filament preparations from Alzheimer's disease brain (Goedert *et al.* 1992*b*). Treatment of filaments with pronase removes a fuzzy coat consisting of N- and C-terminal regions of tau and leaves a pronase-resistant core, which retains the characteristic appearance of the starting filament (Wischik *et al.* 1988). Filaments in extracellular tangles, the remnants of neurofibrillary material left after a tangle-bearing nerve cell has died, show partial proteolysis of the constituent tau protein (Bondareff *et al.* 1990). The proteolytically protected core of the filament contains various fragments of three- and four-repeat tau, consisting of slightly more than three repeats from the microtubule-binding region (Novak *et al.* 1993). The C-shaped subunit seen in the computed cross-section,

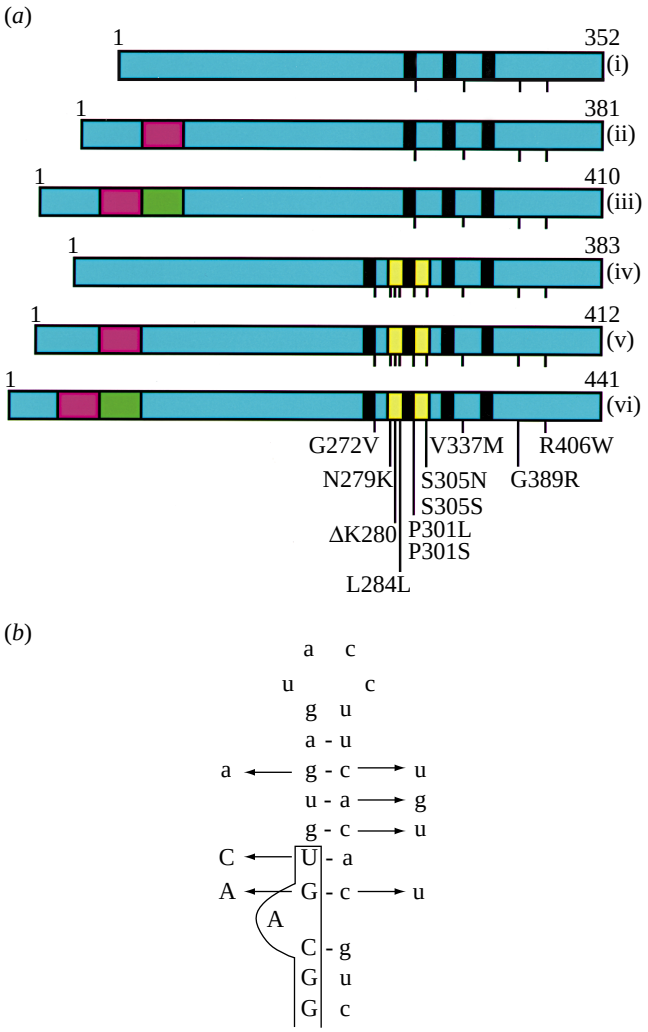

Figure 1. Mutations in the tau gene in frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). (*a*) Schematic diagram of the six tau isoforms (*i–vi*) that are expressed in adult human brain. Alternatively spliced exons are shown in red (exon 2), green (exon 3) and yellow (exon 10); black bars indicate the microtubule-binding repeats. Eight missense mutations, one deletion mutation and two silent mutations in the coding region are shown. Amino-acid numbering corresponds to the 441 amino-acid isoform of human brain tau. (*b*) Stem-loop structure in the pre-mRNA at the boundary between exon 10 and the intron following exon 10. Seven mutations that reduce the stability of this tau exon 10 splicing regulatory element are shown. Exon sequences are shown in upper-case letters and intron sequences in lower-case letters.

which corresponds to the ordered part of the structure, must consist predominantly of this region of tau protein.

Hyperphosphorylation is a major biochemical abnormality of PHF-tau. It is believed to be an early event in the development of the neurofibrillary pathology (Braak *et al.* 1994) and, as a result, tau is unable to interact with microtubules (Bramblett *et al.* 1993; Yoshida & Ihara 1993). Most of the phosphorylated sites are known (Morishima-Kawashima *et al.* 1995; Hanger *et al.* 1998). However, the identity of the protein kinases and/or protein phosphatases that lead to the hyperphosphorylation of tau in the Alzheimer's disease brain remain to be established. It is at present unclear whether hyperphosphorylation of tau is necessary or sufficient for PHF formation.

The vast majority of cases of Alzheimer's disease are sporadic. Nevertheless, there is a small proportion of disease cases that are transmitted as an autosomal-dominant Mendelian trait with high penetrance. The study of these early-onset familial forms of Alzheimer's disease has shown that the abnormal processing of APP plays an important role in the aetiology and pathogenesis of at least some cases of the disease. Less than 10 different missense mutations in the APP gene have been described in approximately 30 families with early-onset Alzheimer's disease (Goate *et al.* 1991). They increase the production of Aβ(1–42), which is believed to be their primary effect (Selkoe 1998). Mutations in the presenilin genes cause the majority of early-onset familial cases of Alzheimer's disease (Sherrington *et al.* 1995). Close to 50 different mutations in the presenilin-1 gene have been described in over 100 families, with a small number of mutations in the presenilin-2 gene in a handful of families. Like mutations in the APP gene, the presenilin gene mutations lead to increased production of Aβ(1–42). They have also been shown to reduce the ability of cells to respond to stress conditions in the endoplasmic reticulum and to increase vulnerability to apoptosis induced by various cellular stresses (Katayama *et al.* 1999; Niwa *et al.* 1999).

In sporadic cases of Alzheimer's disease, neurofibrillary lesions correlate better with the presence of symptoms than do Aβ deposits (Arriagada *et al.* 1992). Moreover, abundant Aβ deposits can be found in some cognitively normal individuals. This observation has provided circumstantial evidence implicating a filamentous tau pathology in the pathogenesis of Alzheimer's disease. The recent discovery of tau gene mutations in familial form of frontotemporal dementia (Poorkaj *et al.* 1998; Hutton *et al.* 1998; Spillantini *et al.* 1998*a*) has shown unambiguously that dysfunction of tau protein can cause neurodegeneration and dementia.

(b) *Frontotemporal dementia and Parkinsonism linked to chromosome 17*

Frontotemporal dementias occur as familial forms and, more commonly, as sporadic diseases. Neuropathologically they are characterized by a remarkably circumscribed atrophy of the frontal and temporal lobes of the cerebral cortex, often with additional, subcortical changes. In 1994, an autosomal dominantly inherited form of frontotemporal dementia with Parkinsonism was linked to chromosome 17q21.2 (Wilhelmsen *et al.* 1994). This observation was followed by the identification of other familial forms of frontotemporal dementia that were linked to this region, resulting in the denomination frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) for this class of disease (Foster *et al.* 1997). All cases of FTDP-17 have so far shown a filamentous pathology made of hyperphosphorylated tau protein, in the absence of Aβ deposits in the majority of cases (Goedert *et al.* 1998; Spillantini *et al.* 2000).

The first mutations in the tau gene in FTDP-17 were reported in 1998 (Poorkaj *et al.* 1998; Hutton *et al.* 1998; Spillantini *et al.* 1998*a*). Currently, close to 20 different mutations have been described in over 40 families with FTDP-17 (figure 1). The mutations fall into different

classes, according to where they lie in the tau gene. They are either missense, deletion or silent mutations in the coding region, or intronic mutations located close to the splice-donor site of the intron following the alternatively spliced exon 10. All known coding region mutations are located in the microtubule-binding region or close to it (figure 1*a*). Mutations in exon 9 (G272V), exon 12 (V337M) and exon 13 (G389R and R406W) affect all six tau isoforms (Poorkaj *et al.* 1998; Hutton *et al.* 1998; Murrell *et al.* 1999). In contrast, mutations in exon 10 (N279K, ΔK280, L284L, P301L, P301S, S305N and S305S) only affect tau isoforms with four microtubule-binding repeats (Hutton *et al.* 1998; Clark *et al.* 1998; Rizzu *et al.* 1999; Iijima *et al.* 1999; Bugiani *et al.* 1999; D'Souza *et al.* 1999; Stanford *et al.* 2000). Most missense mutations reduce the ability of tau to interact with microtubules, as reflected by a marked reduction in the ability of mutant tau to promote microtubule assembly (Hasegawa *et al.* 1998; Hong *et al.* 1998). Moreover, a number of missense mutations have a direct stimulatory effect on tau filament assembly *in vitro* (Nacharaju *et al.* 1999; Goedert *et al.* 1999*b*).

Intronic mutations are located at positions +3, +12, +13, +14 and +16 of the intron following exon 10, with the first nucleotide of the splice-donor site taken as +1 (figure 1*b*) (Hutton *et al.* 1998; Spillantini *et al.* 1998*a*; Yasuda *et al.* 2000). Secondary structure predictions have suggested the presence of an RNA stem-loop structure at the boundary between exon 10 and the intron following exon 10 that is disrupted by the intronic mutations (Hutton *et al.* 1998; Spillantini *et al.* 1998*a*). In addition, the +3 mutation is predicted to lead to increased binding of U1 snRNA to the 5′ splice site (Spillantini *et al.* 1998*a*). Exon trapping experiments have shown that intronic mutations increase splicing in of exon 10 (Hutton *et al.* 1998; D'Souza *et al.* 1999; Varani *et al.* 1999; Grover *et al.* 1999; Yasuda *et al.* 2000). Increased production of transcripts encoding exon 10 has also been demonstrated in brain tissue from patients with tau intronic mutations. This increase is in turn reflected by a change in the ratio of three- to four-repeat isoforms, resulting in a net overproduction of four-repeat isoforms (Spillantini *et al.* 1998*a*; Hong *et al.* 1998; Goedert *et al.* 1999; Hulette *et al.* 1999; Yasuda *et al.* 2000).

The proposed existence of a stem-loop structure at the boundary between exon 10 and the intron following exon 10 received support from the determination of the three-dimensional structure of a 25-nucleotide RNA (extending from positions −5 to +19) by nuclear magnetic resonance spectroscopy (figure 1*b*) (Varani *et al.* 1999). It has been shown that this sequence forms a stable, folded structure. The stem of this tau exon 10 regulatory element consists of a single G−C base pair which is separated from a double helix of six base pairs by an unpaired adenine. The apical loop consists of six nucleotides that adopt multiple conformations in rapid exchange. Known intronic mutations are located in the upper part of the stem. All five mutations reduce the thermodynamic stability of the stem-loop structure, but to various extents. The aminoglycoside antibiotic neomycin has been shown to bind in the major groove of the RNA duplex, where the FTDP-17 mutations cluster (Varani *et al.* 2000). Wild-type and mutant tau exon 10 regulatory elements are stabilized as a result, suggesting that RNA-binding drugs could be able to restore the correct ratio of three- to four-repeat tau isoforms.

The emerging picture is one of missense mutations that lead to a reduced ability of tau to interact with microtubules and to a stimulatory effect on tau filament assembly, and of intronic mutations whose primary effects are at the RNA level, resulting in an overproduction of tau isoforms with four microtubule-binding repeats. However, two missense mutations in exon 10 (N279K and S305N) deviate from this rule in that they do not lead to a reduction in the ability of tau to promote microtubule assembly. Instead, they increase splicing in of exon 10, as is the case of the intronic mutations (Hasegawa *et al.* 1999; D'Souza *et al.* 1999). The N279K mutation (AAT to AAG) in tau creates a splice-enhancer sequence (Clark *et al.* 1998), which explains its effects on exon trapping and on the level of soluble four-repeat tau in brain. Similar results have been obtained with the L284L mutation (CTT to CTC) in exon 10, which is believed to disrupt an exon 10 splicing silencer sequence (D'Souza *et al.* 1999). The ΔK280 mutation (ΔAAG) in exon 10 may also have its primary effect at the RNA level. This work has uncovered the presence of sequence elements within exon 10 that regulate its alternative splicing.

The S305N mutation (AGT to AAT) in tau changes the last amino acid in exon 10 (Iijima *et al.* 1999). This sequence forms part of the predicted stem-loop structure, where the mutation produces a G to A transition at position −1. It is therefore not surprising that the S305N mutation leads to a reduction in the thermodynamic stability of the stem-loop structure and to a marked increase in the splicing in of exon 10 (Hasegawa *et al.* 1999; Varani *et al.* 1999). The silent S305S mutation (AGT to AGC) disrupts the stem-loop structure by virtue of a T to C transition at position 0, explaining its pathogenic effect (Stanford *et al.* 2000). Unlike the −1 and +3 mutations, the mutation at position 0 is not expected to lead to increased binding of U1 snRNA to the 5′ splice site. Besides mutations in the intron following exon 10, additional pathogenic mutations may exist in other introns of the tau gene. Thus, a G to A transition at position +33 of the intron following exon 9 has been described in a patient with frontotemporal dementia (Rizzu *et al.* 1999). This mutation disrupts one of several (A/T)GGG repeats that may play a role in the regulation of the alternative splicing of exon 10.

The different tau gene mutations lead to the formation of tau filaments with various morphologies that can be grouped into distinct categories (figure 2). Some mutations, such as V337M (exon 12) in the Seattle family A, produce filaments (PHFs and SFs) that are identical to those in Alzheimer's disease (Spillantini *et al.* 1996). All six tau isoforms are affected by the mutation and incorporated into the filaments. The tau pathology is neuronal (figure 2*c*). Similar findings have been reported with the R406W (exon 13) mutation (Reed *et al.* 1998; Van Swieten *et al.* 1999).

By contrast, in cases of mutations that affect the splicing in of exon 10, thereby increasing the amount of four-repeat isoforms, the filaments have a quite different ribbon-like morphology. This effect was first reported in multiple system tauopathy with presenile dementia, which was subsequently shown to have a mutation at position

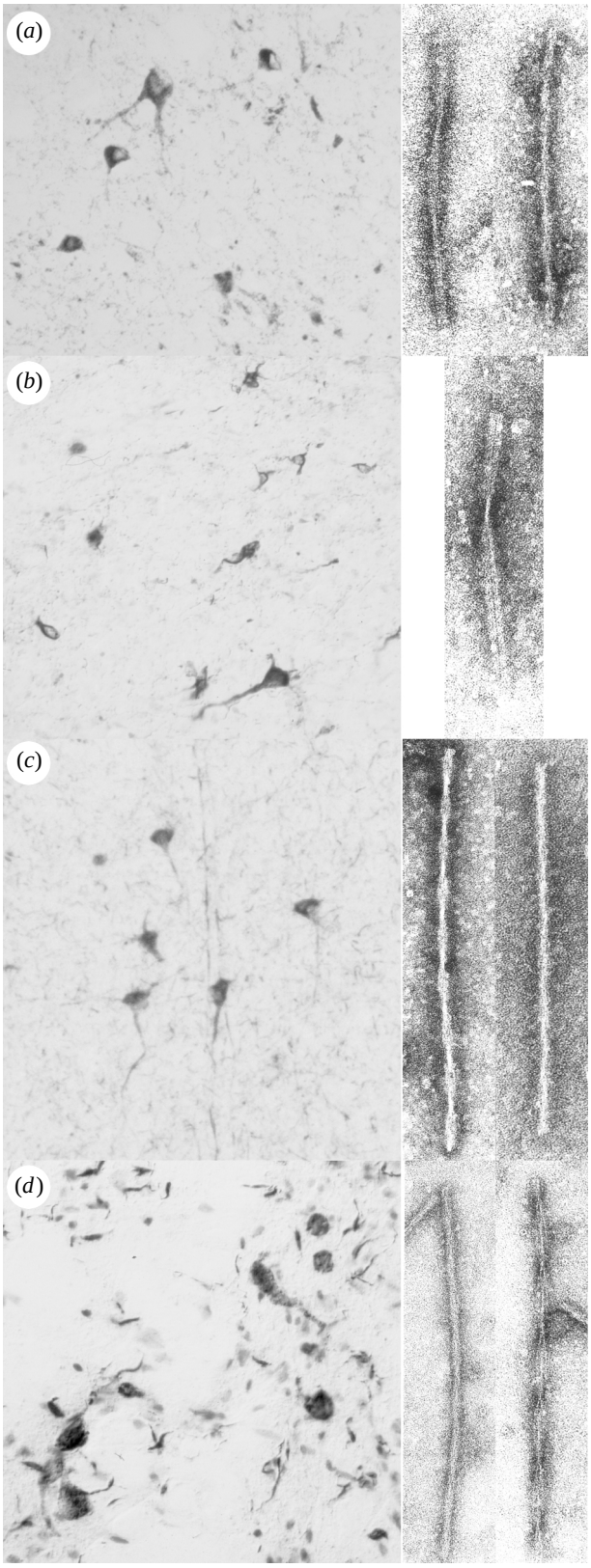

Figure 2. Pathologies of FTDP-17, as revealed by immunohistochemistry for hyperphosphorylated tau protein and the morphologies of isolated tau filaments. (*a*) The P301L mutation in exon 10 gives rise to a neuronal and glial tau pathology. Tau filaments consist of narrow twisted ribbons (left) as the majority species and rope-like filaments (right) as the minority species. They are predominantly made of four-repeat tau isoforms. (*b*) Mutations in the intron following exon 10 give rise to a neuronal and glial tau pathology. (*Cont.*)

+3 in the intron following exon 10 (Spillantini *et al.* 1997*a*, 1998*a*). Identical ribbon-like filaments were found in a family with progressive subcortical gliosis and a mutation at position +16, as well as in a case of frontotemporal dementia-Kumamoto with a mutation at position +12 (Goedert *et al.* 1999*b*; Yasuda *et al.* 2000). The filaments appear as irregularly twisted ribbons with a maximum width of *ca.* 23 nm and a minimum width of *ca.* 5 nm at the position of the twist, corresponding to the thickness of the ribbon (figure 2*b*). A central stain-penetrated dark line is frequently visible and the structure to either side appears as two pale lines of stain-excluding material. Sometimes material on one side of the central line is missing for part of the length of the filament. The transverse ends of the resulting stain-penetrated gaps are usually sharp and show no sign of fraying. These observations indicate an organization of the ribbon based on two equivalent strands of subunits of short axial extent, as for PHFs. Indeed the ribbons closely resemble the untwisted PHFs produced by acid treatment of material from Alzheimer's disease brain (Crowther 1991*b*). The ribbons are decorated by phosphorylation-independent and phosphorylation-dependent anti-tau antibodies and consist of tau with four repeats only. In these cases of FTDP-17 there is abundant tau pathology in both nerve cells and glial cells.

In FTDP-17 cases with a P301L (exon 10) mutation, which affects only four-repeat tau isoforms, two types of filaments are found (figure 2*a*) (Spillantini *et al.* 1998*b*; Mirra *et al.* 1999). The majority species consists of narrow, irregularly twisted, ribbons of maximum width *ca.* 15 nm. The minority species consists of straight filaments *ca.* 12 nm wide with a strongly stranded appearance. Similar stranded filaments are seen at low frequency in some filament preparations from Alzheimer's disease brain. The filaments consist of mainly four-repeat tau and pathology occurs in both nerve cells and glial cells.

A G389R (exon 13) mutation, which affects all six tau isoforms, causes a tauopathy that resembles Pick's disease (figure 2*d*) (Murrell *et al.* 1999). The majority of filaments resemble SFs from Alzheimer's disease. A minority species consists of open-looking, low contrast twisted filaments with a crossover spacing of *ca.* 120 nm and a projected width varying between 6 and 23 nm. In the wider parts, the image shows a strong central white stain-excluding axial line flanked by two somewhat weaker stain-excluding lines. Both kinds of filaments are strongly labelled by anti-tau antibodies and closely resemble the

Figure 2. (*Cont.*) Tau filaments consist of wide twisted ribbons made of four-repeat tau isoforms. The glial pathology is more extensive than in (*a*). (*c*) The V337M mutation in exon 12 gives rise to a neuronal tau pathology. Tau filaments consist of paired helical (left) and straight (right) filaments, like the tau filaments of Alzheimer's disease. They contain all six human brain tau isoforms. Paired helical filaments constitute the majority species. The R406W mutation in exon 13 gives rise to a similar tau pathology. (*d*) The G389R mutation in exon 13 gives rise to a neuronal tau pathology. Tau filaments consist of straight filaments (left) as the majority species and twisted filaments (right) as the minority species. The tau pathology resembles that of Pick's disease. Filaments consist of three-repeat and four-repeat tau isoforms. In all cases tau-containing filaments were identified by labelling with anti-tau antibodies. (Reproduced from Spillantini *et al.* 2000.)

tau filaments of Pick's disease. They contain three- and four-repeat tau isoforms. Neuropathologically, the tau deposits are characterized by large numbers of Pick body-like and axonal inclusions.

The pathway leading from a mutation in the tau gene to neurodegeneration is unknown. The likely primary effect of most missense mutations is a reduced ability of mutant tau to interact with microtubules. It may be equivalent to a partial loss of function, with resultant microtubule destabilization and deleterious effects on cellular processes, such as rapid axonal transport. However, in the case of the intronic mutations and the coding region mutations, whose primary effect is at the RNA level, this appears unlikely. The net effect of these mutations is a simple overproduction of four-repeat tau.

It is possible, however, that a correct ratio of wild-type three- to four-repeat tau is essential for the normal function of tau in human brain. An alternative hypothesis is that a partial loss of function of tau is necessary for setting in motion the mechanisms that will ultimately lead to filament assembly. Earlier work has suggested that three- and four-repeat tau isoforms may bind to different sites on microtubules (Goode & Feinstein 1994). Overproduction of tau isoforms with four repeats may result in an excess of tau over available binding sites on microtubules, thus creating a gain of toxic function similar to that of most missense mutations, with unbound, excess tau available for assembly into filaments.

Where studied, pathological tau from FTDP-17 brain is hyperphosphorylated in a way similar or identical to that found in Alzheimer's disease (Spillantini *et al.* 2000). Hyperphosphorylation is probably an event downstream of the primary effects of the mutations and may be a consequence of the partial loss of function. However, some missense mutations may indirectly affect the phosphorylation state of tau. Thus, in transfected cells, tau protein with the R406W mutation displays only little phosphorylation at T231, S396 and S404, in contrast to wild-type tau and tau with the P301L or V337M mutations (Dayanandan *et al.* 1999; Matsumura *et al.* 1999). Hyperphosphorylation of tau probably reinforces the primary effects of the mutations on microtubule binding and assembly. At present, there is no experimental evidence linking hyperphosphorylation of tau to filament assembly. The mechanisms that lead to assembly of tau into filaments in brain remain to be discovered. It is possible that a reduced ability of tau to interact with microtubules, which could have several different causes, is a necessary step for filament formation. Assembly is an energetically unfavourable, nucleation-dependent process that requires a critical concentration of tau. Many cells may have levels of tau below the critical concentration. Other cells may have effective mechanisms for preventing the formation of tau nuclei, or may be able to degrade them once they have formed.

The significance of the different filament morphologies observed in FTDP-17 is not clear. It is well established that the repeat region of tau forms the densely packed core of the PHF and SF of Alzheimer's disease. Thus, mutations in the repeat region or a change in the relative amounts of three- and four-repeat isoforms could well influence filament morphology. The most important aspect may be the extended filamentous nature of the assemblies and the deleterious effects that this has on intracellular processes, rather than the detailed morphology of the different filaments.

(c) *Other tauopathies*

Filamentous tau deposits are also a characteristic of several other, mostly sporadic, neurodegenerative diseases, where they are found in the absence of Aβ deposits. They include Pick's disease, progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) (Buée & Delacourte 1999).

Pick's disease was the first frontotemporal dementia to be described (Pick 1892). Neuropathologically, it is characterized by the presence of Pick bodies (Alzheimer 1911), which stain with anti-tau antibodies (Pollock *et al.* 1986). The filaments seen in sectioned Pick bodies are mostly 15 nm straight filaments, with a smaller number of twisted filaments (Rewcastle & Ball 1968; Murayama *et al.* 1990). Biochemically, these filaments are said to contain predominantly, if not exclusively, three-repeat tau isoforms (Delacourte *et al.* 1996). FTDP-17 cases with the G272V and G389R mutations in tau closely resemble Pick's disease clinically and neuropathologically (Schenk 1959; Hutton *et al.* 1998; Spillantini *et al.* 1998*b*; Murrell *et al.* 1999).

The finding that overproduction of four-repeat tau leads to its assembly into twisted ribbons and causes disease may shed light on the pathogenesis of PSP and CBD. Neuropathologically, both diseases are characterized by a neuronal and glial tau pathology, with the tau filaments comprising only four-repeat tau isoforms (Flament *et al.* 1991; Ksiezak-Reding *et al.* 1994; Sergeant *et al.* 1999). The predominant form of filament seen in sectioned material from PSP brain is straight, with a width of *ca.* 15 nm (Tomonaga 1979), but PHF-like filaments (Yagashita *et al.* 1979) and wider twisted ribbons (Takauchi *et al.* 1983) have also been reported. Isolated filaments from CBD brain have a greater width and a longer, more irregular twist than PHFs, appearing similar to the twisted ribbons described above in tau splicing diseases. A recent study has reported an increase in exon 10-containing transcripts in brainstem in PSP (Chambers *et al.* 1999). It remains to be seen whether this is reflected in increased levels of soluble four-repeat tau isoforms.

An association between PSP and homozygosity of a common allele at a dinucleotide repeat in the intron following exon 9 of the tau gene has been described (Conrad *et al.* 1997). More recently, two common tau haplotypes that differ at the nucleotide level, but not at the level of the protein coding sequence, have been reported (Baker *et al.* 1999). Homozygosity of the more common allele H1 appears to predispose to PSP. Taken together, this work suggests that PSP may be caused by an imbalance between three- and four-repeat tau isoforms, analogous to the FTDP-17 cases with mutations that affect splicing in of exon 10. Interestingly, an individual with a mutation at position 0 of the stem-loop structure at the boundary between exon 10 and the intron following exon 10 presented with the clinical and neuropathological picture of PSP (Stanford *et al.* 2000). It remains to be seen whether CBD is also caused by an imbalance between three- and four-repeat tau isoforms. It

is noteworthy that an individual with the P301S mutation in tau presented with a clinical diagnosis of CBD (Bugiani *et al.* 1999).

4. SYNTHETIC TAU FILAMENTS

The availability of large quantities of recombinant tau isoforms and the ease with which tau fragments can be expressed have facilitated studies aimed at producing synthetic tau filaments. It has been shown that paired helical-like filaments can be assembled *in vitro* from bacterially expressed non-phosphorylated three-repeat fragments of tau. Filaments with dimensions similar to Alzheimer PHFs were produced by hanging-drop equilibration of the fragments against high concentrations of Tris at acidic pH (Crowther *et al.* 1992; Wille *et al.* 1992). These findings lent strong support to the view that the repeat region of tau is the only component necessary for the morphological appearance of the PHF. They also suggested that the N- and C-terminal regions of tau are inhibitory towards self-assembly. However, they failed to provide any insight into filament formation *in vivo*, since they were only obtained with truncated tau under non-physiological conditions. This contrasts with dispersed tau filaments from Alzheimer's disease or FTDP-17 brain that consist of full-length tau protein (Goedert *et al.* 1992*b*).

During the course of experiments using sulphated glycosaminoglycans to stimulate phosphorylation of full-length recombinant tau, we noticed the presence of high-molecular weight aggregates of tau that formed in a glycosaminoglycan-dependent, but phosphorylation-independent, manner (Goedert *et al.* 1996*b*; Hasegawa *et al.* 1997). Electron microscopy revealed the presence of many filaments, with differing morphologies for tau isoforms with three or four microtubule-binding repeats (figure 3). Tau isoforms with three repeats gave filaments with a typical paired helical-like morphology, when incubated with heparin or heparan sulphate, whereas tau isoforms with four repeats gave filaments with a straight appearance. By immunoelectron microscopy, the paired helical-like filaments were decorated by antibodies directed against the N- and C-termini of tau, but not by an antibody against the microtubule-binding repeat region. These results, which indicate that in the filaments the repeat region of tau is inaccessible to the antibody, are identical to those previously obtained with PHFs from Alzheimer's disease brain. The dimensions of tau filaments formed in the presence of sulphated glycosaminoglycans are also similar to those of filaments extracted from Alzheimer's disease brain, with a diameter of *ca.* 20 nm for twisted and 15 nm for straight filaments, with a crossing-over spacing of *ca.* 80 nm for paired helical-like filaments, although their twist is in general less regular than in Alzheimer filaments. Assembly of tau into filaments in the presence of sulphated glycosaminoglycans occurs after a lag period and is heavily concentration dependent, consistent with a nucleation-dependent process (Goedert *et al.* 1996*b*; Pérez *et al.* 1996; Friedhoff *et al.* 1998). Subsequent to this work, RNA (Kampers *et al.* 1996; Hasegawa *et al.* 1997) and arachidonic acid (Wilson & Binder 1997; King *et al.* 2000) were also shown to induce the bulk assembly of full-length recombinant tau protein into filaments. These findings have provided robust methods for the assembly of full-length tau into filaments. Pathological co-localization of sulphated glycosaminoglycans and hyperphosphorylated tau protein has suggested that this work may also be relevant for the assembly of tau in Alzheimer's disease brain (Snow *et al.* 1989; Goedert *et al.* 1996*b*).

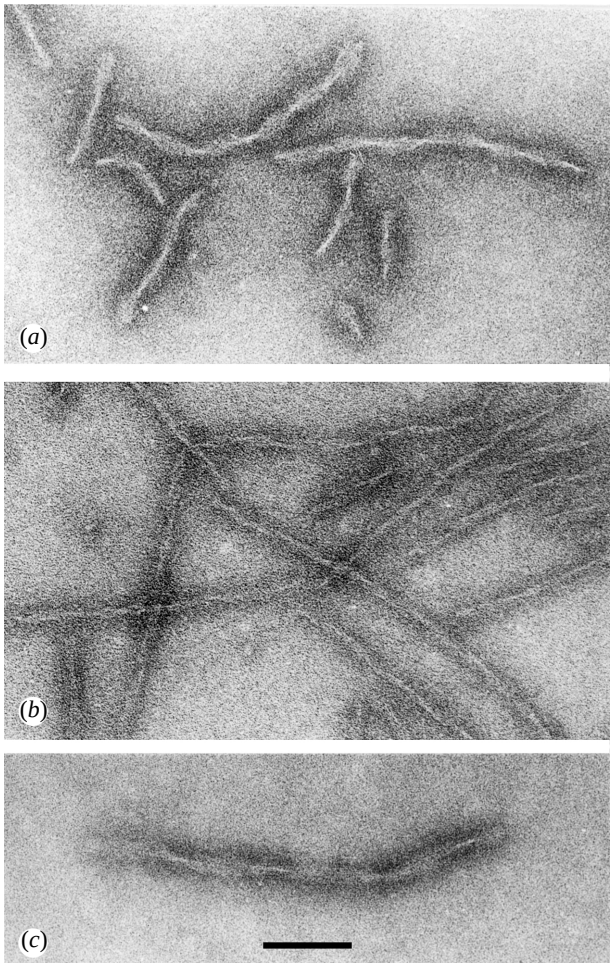

Figure 3. Sulphated glycosaminoglycan-induced filament assembly of human tau protein. (*a*) The three-repeat containing 381 amino-acid isoform (htau37) or (*b*) the four-repeat containing 441 amino-acid isoform (htau40) was incubated with heparin. (*c*) htau37 was phosphorylated with neuronal cdc2-like kinase before the addition of heparin. Note the presence of paired helical-like filaments in (*a*) and (*c*) and of straight filaments in (*b*). Scale bar, 100 nm. (Reproduced from Goedert *et al.* 1996*b*.)

5. THE SYNUCLEIN FAMILY

Synucleins are abundant brain proteins whose physiological functions are poorly understood (Maroteaux *et al.* 1988; Uéda *et al.* 1993; Jakes *et al.* 1994; Ji *et al.* 1997; Buchman *et al.* 1998*a*). In humans, at least three different members (called α-, β- and γ-synuclein) of this family are expressed from three different genes. They range from 127 to 140 amino acids in length and are 55–62% identical in amino-acid sequence, with a similar domain organization. The N-terminal half of each protein is taken up by imperfect amino-acid repeats, with the consensus sequence KTKEGV (figure 4). Individual repeats are

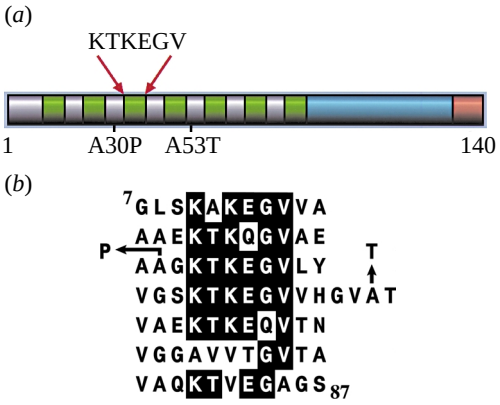

Figure 4. Mutations in the α-synuclein gene in familial Parkinson's disease. (*a*) Schematic diagram of human α-synuclein. The repeats with the consensus sequence KTKEGV are shown as green bars. The hydrophobic region is shown in blue and the negatively charged C-terminus in red. The two known missense mutations are indicated. (*b*) Repeats in human α-synuclein. Residues 7–87 of the 140 residue protein are shown. Amino-acid identities between at least five of the seven repeats are indicated by black bars. The Ala to Pro mutation at residue 30 between repeats two and three and the Ala to Thr mutation at residue 53 between repeats four and five are shown.

separated by an inter-repeat region of five to eight amino acids. Depending on the alignment, α-synuclein has five to seven repeats, whereas β- and γ-synuclein have five repeats each. The repeats are followed by a hydrophobic middle region and a negatively charged C-terminal region (figure 4). α- and β-synuclein have an identical C-terminus. By immunohistochemistry, they are concentrated in nerve terminals, with little staining of nerve cell bodies and dendrites (Jakes *et al.* 1994). Ultrastructurally, they are found in nerve terminals, in close proximity to synaptic vesicles (Clayton & George 1999). In contrast, γ-synuclein appears to be present throughout nerve cells (Buchman *et al.* 1998*b*).

Experimental studies have shown that α-synuclein can bind to lipid membranes through its amino-terminal repeats, suggesting that it may be a lipid-binding protein (Davidson *et al.* 1998; Jensen *et al.* 1998; McLean *et al.* 2000). α- and β-synuclein have been shown to selectively inhibit phospholipase D2 (Jenco *et al.* 1998). This isoform of phospholipase D localizes to the plasma membrane, where it may play a role in signal-induced cytoskeletal regulation and endocytosis. It is therefore possible that α- and β-synuclein regulate vesicular transport processes. Little is known about post-translational modifications of synucleins in brain. In transfected cells, α-synuclein becomes constitutively phosphorylated at serine residues 87 and 129 (Okochi *et al.* 2000). Inactivation of the α-synuclein gene by homologous recombination has been shown to lead to increased release of dopamine with paired stimuli, suggesting that α-synuclein may be an activity-dependent, negative regulator of neurotransmission (Abeliovich *et al.* 2000). The synuclein sequences from several vertebrate species are very similar. No synuclein homologues have been identified in *S. cerevisiae*, *C. elegans* or *D. melanogaster*, suggesting that the presence of synucleins may be limited to vertebrates. Synucleins are believed to be natively unfolded proteins with little ordered secondary structure (Weinreb *et al.* 1996; Serpell *et al.* 2000). They are resistant to heat and dilute acid, in keeping with their extended conformation (Jakes *et al.* 1994).

6. DISEASES WITH α-SYNUCLEIN PATHOLOGY

(a) *Parkinson's disease*

Parkinson's disease is defined by the presence of Lewy bodies and Lewy neurites in the substantia nigra and several other brain regions. Brainstem Lewy bodies appear as round, intracytoplasmic inclusions with a dense eosinophilic core and a clearer surrounding corona (Lewy 1912). Ultrastructurally, they are composed of a core of filamentous and granular material that is surrounded by radially oriented filaments 10–20 nm in diameter (Duffy & Tennyson 1965; Forno 1996). Lewy neurites constitute an important component of the pathology of Parkinson's disease. They correspond to abnormal neurites that have the same immunohistochemical staining profile as Lewy bodies and consist ultrastructurally of abnormal filaments similar to those in Lewy bodies. Despite much work, the biochemical nature of the Lewy body filament remained unknown until recently.

Most cases of Parkinson's disease are idiopathic, without an obvious family history. However, a small percentage of cases is familial and inherited in an autosomal-dominant manner (Polymeropoulos *et al.* 1997; Krüger *et al.* 1998). Two separate missense mutations have been discovered in the α-synuclein gene in kindreds with early-onset familial Parkinson's disease (figure 4). The first mutation, which changes residue 53 in α-synuclein from alanine to threonine (A53T), was identified in a large Italian–American kindred and three smaller Greek pedigrees. The second mutation, which changes alanine-30 to proline (A30P), was found in a German pedigree with early-onset Parkinson's disease.

The identification of the A53T mutation as the cause of early-onset Parkinson's disease in a small number of families was quickly followed by the discovery that α-synuclein is the major component of Lewy bodies and Lewy neurites (Spillantini *et al.* 1997*b*). Full-length, or close to full-length α-synuclein has been found in Lewy bodies and Lewy neurites, with both the core and the corona of the Lewy body being stained (Giasson *et al.* 2000). No staining of pathological structures has been observed using antibodies specific for β- or γ-synuclein (Spillantini *et al.* 1998*c*).

(b) *Dementia with Lewy bodies*

Lewy bodies and Lewy neurites are also the defining neuropathological characteristics of dementia with Lewy bodies, a common late-life dementia that exists in a pure form or overlaps with the neuropathological characteristics of Alzheimer's disease, especially Aβ deposits. Unlike Parkinson's disease, it is characterized by the presence of numerous Lewy bodies and Lewy neurites in the cerebral cortex (Kosaka 1978). As in Parkinson's disease, the Lewy body pathology is also present in the substantia nigra and other subcortical regions. Lewy bodies and Lewy neurites in dementia with Lewy bodies are strongly

immunoreactive for α-synuclein, exactly as in the pathological structures of Parkinson's disease (Spillantini *et al.* 1997*b*). The Lewy body pathology that is sometimes associated with other neurodegenerative diseases, such as sporadic and familial Alzheimer's disease, Down's syndrome and neurodegeneration with brain iron accumulation type I (Hallervorden-Spatz syndrome) has also been shown to be α-synuclein positive (Lippa *et al.* 1998, 1999; Tu *et al.* 1998; Spillantini *et al.* 1999).

Isolated filaments extracted from brains of patients with dementia with Lewy bodies were strongly labelled for α-synuclein, demonstrating that they contain α-synuclein as a major component (Spillantini *et al.* 1998*c*; Serpell *et al.* 2000). The labelled structures showed various morphologies, including a 5 nm wide filament, a somewhat less regular, 10 nm wide filament with a dark stain-penetrated centre line, a 10 nm filament with slender 5 nm extensions at one or both ends and a twisted 5–10 nm filament with a crossover spacing of about 90 nm. The majority of filaments were 10 nm wide. Whereas antibodies directed against the C-terminal half of α-synuclein labelled filaments along their entire lengths, an antibody specific for the N-terminal region labelled only one end of each filament. This indicates that the N-terminal region of α-synuclein is buried in the body of the filament and exposed only at one end. It also suggests that the filaments are polar structures.

(c) *Multiple system atrophy*

Following the discovery of α-synuclein in the filamentous lesions of Lewy body diseases, multiple system atrophy was shown to be characterized by filamentous α-synuclein deposits. Clinically, it comprises cases of olivopontocerebellar atrophy, Shy–Drager syndrome and striatonigral degeneration. Neuropathologically, glial cytoplasmic inclusions, which consist of filamentous aggregates, are the defining feature of multiple system atrophy (Papp *et al.* 1989). They are found mostly in the cytoplasm and, to a lesser extent, in the nucleus of oligodendrocytes. Inclusions are also observed in the cytoplasm and nucleus of some nerve cells, as well as in neuropil threads.

Glial cytoplasmic inclusions are strongly immunoreactive for α-synuclein (Wakabayashi *et al.* 1998*a*,*b*; Mezey *et al.* 1998; Spillantini *et al.* 1998*d*; Gai *et al.* 1998; Tu *et al.* 1998; Arima *et al.* 1998) and filaments isolated from the brains of patients with multiple system atrophy are strongly labelled by α-synuclein antibodies (Spillantini *et al.* 1998*d*). The filament morphologies and their staining characteristics were found to be similar to those of filaments extracted from the brains of patients with dementia with Lewy bodies. Two distinct filament morphologies were observed. Some filaments showed a distinctly twisted appearance alternating in width between 5 and 18 nm, with a period of 70–90 nm. The other class of filament gave images with a more uniform width of *ca.* 10 nm. This work has revealed an unexpected molecular link between multiple system atrophy and the Lewy body diseases, Parkinson's disease and dementia with Lewy bodies.

7. SYNTHETIC α-SYNUCLEIN FILAMENTS

The discovery of α-synuclein in the filamentous lesions of Parkinson's disease, dementia with Lewy bodies and multiple system atrophy led to attempts at producing synthetic α-synuclein filaments from expressed wild-type and mutant proteins. An initial study showed that human α-synuclein lacking the last 20 amino acids readily assembled into filaments with morphologies and staining characteristics similar to those of the disease filaments (Crowther *et al.* 1998). This study indicated that the packing of α-synuclein molecules in the filaments *in vitro* is very similar to that of filaments extracted from brain. It provided a strong argument in favour of the view that the disease filaments are made of α-synuclein.

Subsequently, full-length α-synuclein was also shown to assemble into filaments, and the A53T mutation was found to increase the rate of filament assembly (figure 5) (Conway *et al.* 1998; El-Agnaf *et al.* 1998; Narhi *et al.* 1999; Giasson *et al.* 1999; Serpell *et al.* 2000). The effect of the

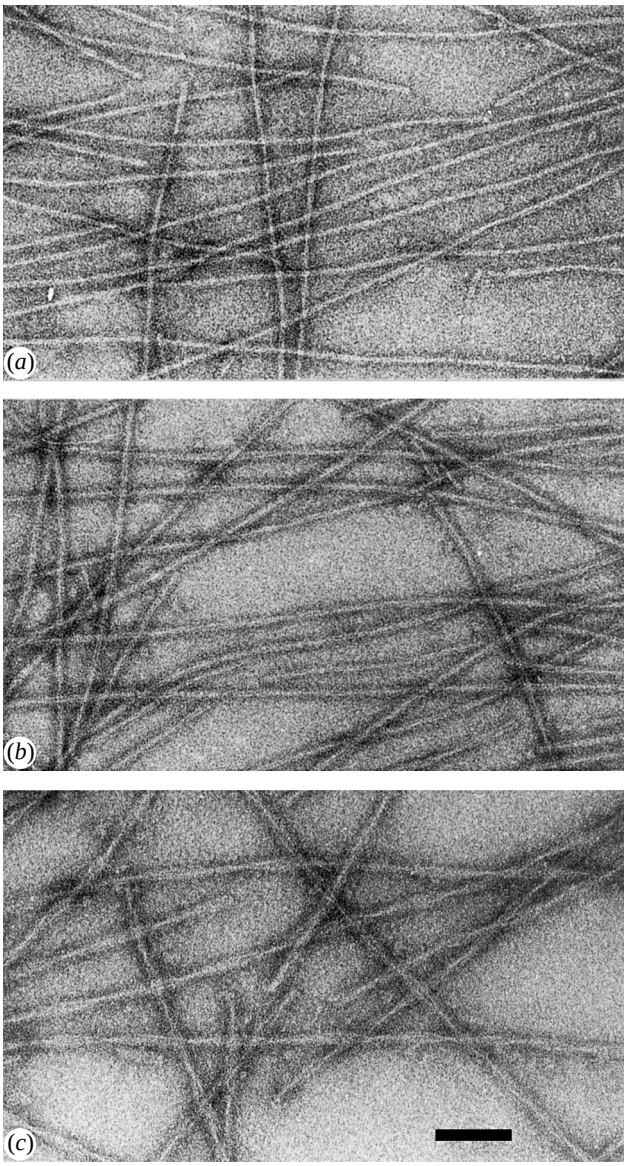

Figure 5. Electron micrographs of synthetic α-synuclein filaments. Filaments were assembled from (*a*) recombinant wild-type human α-synuclein, (*b*) human A30P α-synuclein and (*c*) human A53T α-synuclein. Note the predominantly straight filaments in (*a*,*b*) and the predominantly twisted filaments in (*c*). Scale bar, 100 nm. (Reproduced from Serpell *et al.* 2000.)

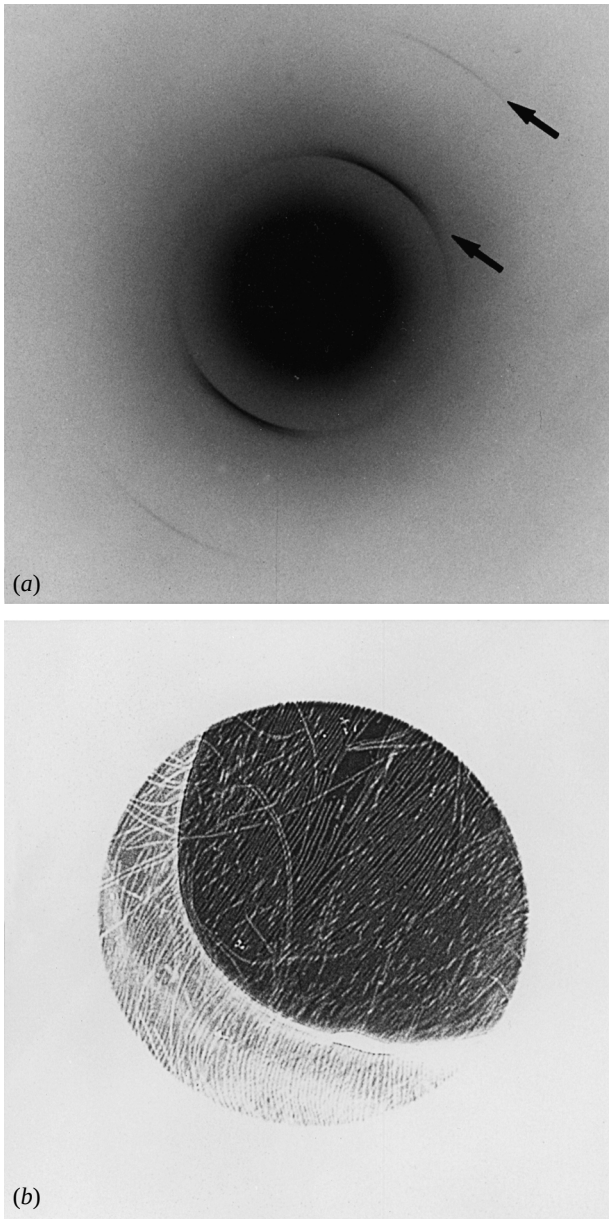

Figure 6. Electron diffraction from hydrated wild-type human α-synuclein synthetic filaments. (*a*) Electron diffraction pattern with orientated arcs at 0.47 nm and 0.235 nm indicated by arrows. (*b*) Image of diffracting area showing partially orientated filaments with axes running roughly perpendicular to the arcs in (*a*), indicating cross-β structure. The illuminated area is *ca.* 1 μm in diameter. (Reproduced from Serpell *et al.* 2000.)

A30P mutation on filament assembly is less clear (figure 5) (Narhi *et al.* 1999; Conway *et al.* 2000*a*; Serpell *et al.* 2000). This mutation has been shown to produce reduced binding of α-synuclein to rat brain vesicles *in vitro*, suggesting that this may be its primary effect (Jensen *et al.* 1998). The assembly of α-synuclein is a nucleation-dependent process (Wood *et al.* 1999) that is accompanied by the transition from a random coil to a β-pleated sheet conformation (Conway *et al.* 2000*a*,*b*; Serpell *et al.* 2000). By X-ray fibre diffraction and electron diffraction, α-synuclein filaments exhibit a meridional 0.47 nm reflection diagnostic of the cross-β conformation that is the defining characteristic of amyloid (figure 6) (Serpell *et al.* 2000). Under the conditions of these experiments, β- and γ-synuclein failed to assemble into filaments and remained in a random coil conformation (Serpell *et al.* 2000). This behaviour is consistent with their absence from the filamentous lesions of the α-synuclein diseases (Spillantini *et al.* 1998*c*).

8. CONCLUSION

The discovery that tau protein and α-synuclein account for the filamentous deposits of most cases of late-onset neurodegenerative disease has provided a general underlying theme to the study of these disorders. The presence of tau gene mutations in FTDP-17 and of α-synuclein gene mutations in familial Parkinson's disease has shown that dysfunction of tau and α-synuclein proteins can cause neurodegeneration. The development of *in vitro* methods for filament formation from expressed tau and α-synuclein has provided first insights into mechanisms governing assembly.

However, the mechanisms by which dysfunction of tau and α-synuclein proteins leads to neurodegeneration remain to be elucidated. Their unravelling will depend on the development of experimental animal models for tauopathies and α-synucleinopathies. Much work is currently being directed towards this objective and the first findings are encouraging (Ishihara *et al.* 1999; Spittaels *et al.* 1999; Probst *et al.* 2000; Masliah *et al.* 2000). So far, the best experimental model for Parkinson's disease is one where expression of wild-type or mutant human α-synuclein in nerve cells of *D. melanogaster* leads to the formation of filamentous inclusions that resemble Lewy bodies (Feany & Bender 2000). Dopaminergic cells with these inclusions were found to undergo degeneration. The genomic sequence of *D. melanogaster* has provided no evidence for a synuclein homologue (Rubin *et al.* 2000). Compared with transgenic mice, *D. melanogaster* forms filaments from expressed human α-synuclein with relative ease and speed, suggesting that vertebrates may have evolved mechanisms that prevent the assembly of α-synuclein. Disruption of these so far unknown mechanisms could well play an important role in the development of sporadic α-synucleinopathies.

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Discussion

M. Perutz (*Medical Research Council Laboratory of Molecular Biology, Cambridge, UK*). Is there any hint as to why the tau protein forms these filamentous assemblies?

M. Goedert. No. We do not yet understand the mechanisms that lead to the assembly of wild-type tau protein into filaments in the brain. We need animal models to study this.

J. Masel (*Department of Zoology, University of Oxford, UK*). You said that the ratio of tau isoforms has to be perturbed to get fibril formation. Could this be related to the idea that heterologous forms of PrP can block fibril extension? Do you see interference *in vitro* if you add the different tau isoforms?

M. Goedert. In those cases where there is a relative overproduction of four-repeat tau, the filament contains only four-repeat tau, not three-repeat tau. On the other hand in many other cases with tau mutations, as well as in Alzheimer's disease, all six tau isoforms are present. We have done a limited number of *in vitro* experiments mixing three- and four-repeat tau. There is a slight inhibitory effect, with the morphology of the filaments looking like three-repeat tau. You get a smaller number of these types of filament than when you have three-repeat tau by itself.

R. Seckler (*Physik. Biochemie., University of Potsdam, Germany*). Do you have any clue as to why β- and γ-synuclein, in contrast to α-synuclein, will not form these fibrils?

M. Goedert. Not in any precise terms; they are about 60% identical in sequence and the repeat region is very similar. The C-terminal half is less well conserved. When you have truncation of the C-terminal half you get increased rates of assembly of the α-synuclein, so I would imagine that in the β- and γ-synuclein there is increased inhibition by the C-terminal half. We are addressing this by making hybrids.

C. M. Dobson (*Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, UK*). It seems reasonable that proteins that are natively unfolded should be able to aggregate into fibrils easily. Are many brain proteins natively unfolded?

M. Goedert. I do not know how many brain proteins are natively unfolded. If you use heat stability as a criterion, there are not a lot of proteins left in soluble form in a whole brain extract after boiling.

C. M. Dobson. Is the functional role of unfolded proteins understood?

M. Goedert. Not in a general sense. Tau is natively unfolded on its own in solution. What state it is in when bound to microtubules is not known, but it may become structured. The α-synuclein is similar—it probably binds to something through the repeat regions and may become structured.

M. B. Pepys (*Department of Medicine, Hammersmith Hospital, London, UK*). Would you speculate as to what the connection might be between Alzheimer's disease caused by APP and presenilin mutations and tau pathology?

M. Goedert. In familial cases of Alzheimer's disease the mutations in APP and presenilin are primary, but it may be tau pathology that causes neurodegeneration. How these mutations lead to the tau pathology is unknown.

M. Joniau (*University of Leuven, Belgium*). As far as I recall, tau dissociates from microtubules after phosphorylation. This needs a cellular signal. Is the reason why tau polymerizes to form tangles not simply caused by a defect in the signal that then leads to excessive phosphorylation?

M. Goedert. Signalling is not well understood. Tau is clearly hyperphosphorylated in all these diseases. In mutated tau the protein gets phosphorylated in a similar way to what you see in Alzheimer's disease. So it is likely that phosphorylation is downstream of the primary effects. With one possible exception none of the known missense mutations creates additional phosphorylation sites.