CHAPTER

47

Synucleinopathies and Tauopathies

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INTRODUCTION

The salient histological features of the most common neurodegenerative diseases were described over a century ago (Goedert & Spillantini, 2006). In 1907, Alois Alzheimer in Munich and Oskar Fischer in Prague described neuritic plaques and neurofibrillary tangles in the disease that Emil Kraepelin, head of the Munich Institute, named after Alzheimer three years later. In 1911, Alzheimer described argyrophilic inclusions as the characteristic neuropathological lesion of a form of lobar degeneration that was subsequently named Pick's disease, after Arnold Pick, Head of the Department of Neuropsychiatry of the German University in Prague, where Fischer worked (the inclusions are now called Pick bodies). In 1912, while working in Alzheimer's laboratory in Munich, Friedrich Lewy described the inclusions characteristic of Parkinson's disease (these inclusions are now called Lewy bodies.) In the 1960s, these inclusions were shown to be made of abnormal filaments. Over the past 30 years, a direct correspondence between inclusion formation and the degenerative process has emerged.

TABLE 47.1 Synucleinopathies

Parkinson's disease
Dementia with Lewy bodies
Pure autonomic failure
REM sleep behavior disorder
Lewy body dysphagia
Incidental Lewy body disease
Inherited Lewy body diseases
Multiple system atrophy

TABLE 47.2 Tauopathies

Alzheimer's disease
Down syndrome
Progressive supranuclear palsy
Corticobasal degeneration
Pick's disease
Argyrophilic grain disease
Tangle-only dementia
Chronic traumatic encephalopathy
ALS/parkinsonism-dementia complex of Guam
Non-Guamanian ALS with tangles
Guadeloupean parkinsonism
Frontotemporal dementia and parkinsonism linked to chromosome 17T
Postencephalitic parkinsonism
Subacute sclerosing panencephalitis
Pantothenate kinase-associated neurodegeneration
Niemann-Pick disease type C
Familial British dementia
Familial Danish dementia

This was made possible by the coming together of two independent lines of research. On the one hand, the biochemical study of the neuropathological lesions resulted in the identification of their major molecular components. On the other hand, the study of rare, familial forms of Alzheimer's disease (AD), frontotemporal dementia (FTD) and Parkinson's disease (PD) led to the identification of gene defects that cause inherited forms of disease. Remarkably, the defective genes were found to encode or increase the expression of the main components of the neuropathological lesions. It therefore appears that a toxic property conferred by these mutations causes disease. A similar toxic property may also underlie the sporadic forms of disease.

Lewy bodies, neurofibrillary tangles and Pick bodies are intracellular filamentous inclusions. Lewy bodies are made of the protein α-synuclein, whereas neurofibrillary tangles and Pick bodies are made of the microtubule-associated protein tau. Mutations in the α-synuclein gene (*SNCA*) or an increase in copy number cause dominantly inherited forms of PD and dementia with Lewy bodies (DLB). Mutations in the tau gene (*MAPT*) give rise to an inherited form of FTD with parkinsonism. Synucleinopathies and tauopathies account for the majority of cases of late-onset neurodegenerative disease (Tables 47-1 and 47-2).

SYNUCLEINS

The human synuclein family consists of three members

α-Synuclein, β-synuclein and γ-synuclein range from 127 to 140 amino acids in length, and are 55–62% identical in sequence, with a similar domain organization (Figure 47-1A). They are encoded by three genes located on chromosomes 4q23 (*SNCA*), 5q35 (*SNCB*) and 10q21 (*SNCC*). More than half of each protein is taken up by six or seven imperfect 11-amino-acid repeats with the consensus sequence KTKEGV. This positively charged region is followed by a hydrophobic middle part and a negatively charged carboxy-terminal region. By immunohistochemistry, α- and β-synucleins are abundant and concentrated in nerve terminals, with little staining of cell bodies and dendrites. Ultrastructurally, they are found in close proximity to synaptic vesicles. In contrast, γ-synuclein is present throughout nerve cells in many brain regions. In rat, α-synuclein is most abundant in telencephalon and diencephalon, with lower levels in more caudal regions. β-Synuclein is distributed throughout the central nervous system, whereas γ-synuclein is most abundant in midbrain, pons, spinal cord and peripheral nervous system, with lower levels in forebrain.

Synucleins are lipid-binding proteins

For a long time, synucleins were believed to have little ordered structure. However, recent work has shown that native α-synuclein is a homotetramer with a predominantly α-helical conformation. Synucleins have only been identified in vertebrates. Experimental studies have shown that α-synuclein binds to lipid membranes (Davidson et al., 1998). Monomeric α-synuclein adopts structures rich in α-helical character upon binding to lipid membranes containing acidic phospholipids. This conformation is taken up by amino acids 1–98, with residues 99–140 being unstructured. Tetrameric α-synuclein has a higher lipid-binding affinity than monomeric protein. In cell lines and primary neurons treated with fatty acids, α-synuclein accumulates on phospholipid monolayers surrounding triglyceride-rich droplets. β-Synuclein binds in a similar way, but γ-synuclein fails to bind and remains cytosolic. Synucleins are phosphoproteins, with serine and tyrosine phosphorylation having been observed in transfected cells. It remains to be established whether phosphorylation of synucleins plays a physiological role in brain. α-synuclein is degraded by the proteasome. A role for the autophagy-lysosome system has also been suggested. Inactivation of *SNCA* results in mice that are largely normal. However, the knockout of all three synucleins results in age-dependent neurological impairment, decreased

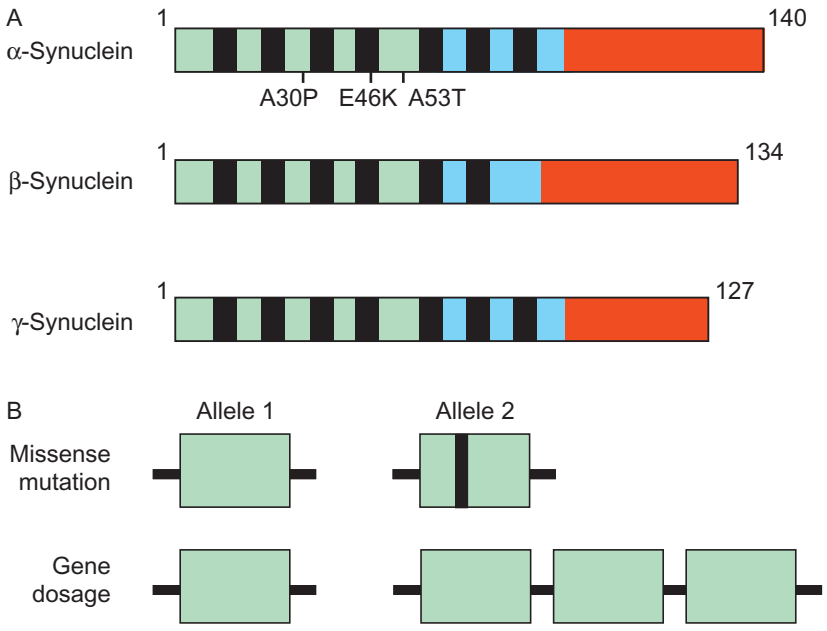


FIGURE 47-1 **(A), Diagram of the three human synucleins, which range from 127 to 140 amino acids in length.** The amino-terminal repeats are shown as black bars. Positively charged regions are indicated in green, hydrophobic regions in blue and negatively charged regions in red. Missense mutations (A30P, E46K, A53T) in α-synuclein, which cause Parkinson's disease and dementia with Lewy bodies, are shown. **(B)**, Missense mutations in *SNCA* or an increase in gene dosage (duplication or triplication, with a triplication shown here) of the chromosomal region containing *SNCA* cause autosomal dominant inherited forms of Parkinson's disease and dementia with Lewy bodies. *SNCA* is shown schematically in green.

assembly of SNARE complexes and premature death (Burré et al., 2010).

PARKINSON'S DISEASE AND OTHER LEWY BODY DISEASES

SNCA mutations cause familial Parkinson's disease

In 1997, a missense mutation (A53T) in *SNCA* was shown to cause disease in a dominantly inherited form of PD with Lewy body pathology (Polymeropoulos et al., 1997). Subsequently, two additional missense mutations (A30P and E46K) were identified in families with PD and DLB. All three mutations are located in the repeat region of α-synuclein. Overexpression of wild-type α-synuclein has also been identified as a cause of PD and DLB (Figure 47-1B) (Singleton et al., 2003). Curiously, the amino acid at position 53 in mouse and rat α-synuclein is a threonine, not an alanine, suggesting that it is not simply the presence of a threonine at position 53 that is pathogenic, but instead some difference in conformation of mutant protein that is characteristic of human, but not rodent α-synuclein.

Lewy body filaments are made of α-synuclein

In 1997, Lewy bodies and Lewy neurites from PD and DLB were shown to be immunoreactive for α-synuclein (Figure 47-2) (Spillantini et al., 1997). DLB is characterized by large numbers of Lewy bodies and Lewy neurites in cortical brain areas, in addition to the substantia nigra. Filaments are unbranched, with a length of 200–600 nm and a width of 5–10 nm. The core of the filament extends over 70 amino acids and overlaps with the lipid-binding region of α-synuclein. Filaments have a cross-β structure like other amyloid fibers.

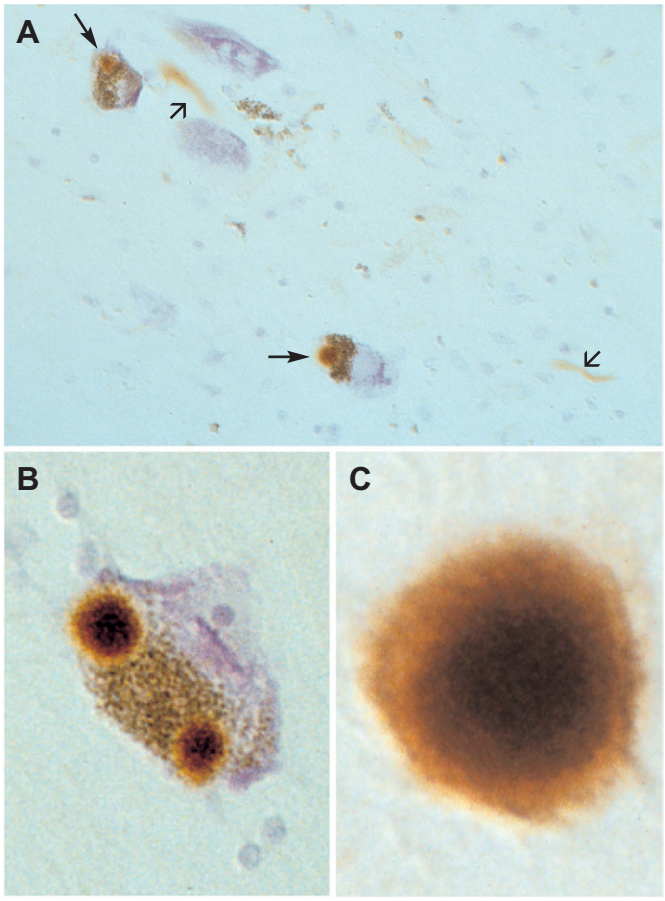


FIGURE 47-2 **Substantia nigra from patients with Parkinson's disease immunostained for α-synuclein. (A)**, Two pigmented nerve cells, each containing an α-synuclein-positive Lewy body. Lewy neurites (small arrows) are also immunopositive. **(B)**, Pigmented nerve cell with two α-synuclein-positive Lewy bodies. **(C)**, α-Synuclein-positive extracellular Lewy body.

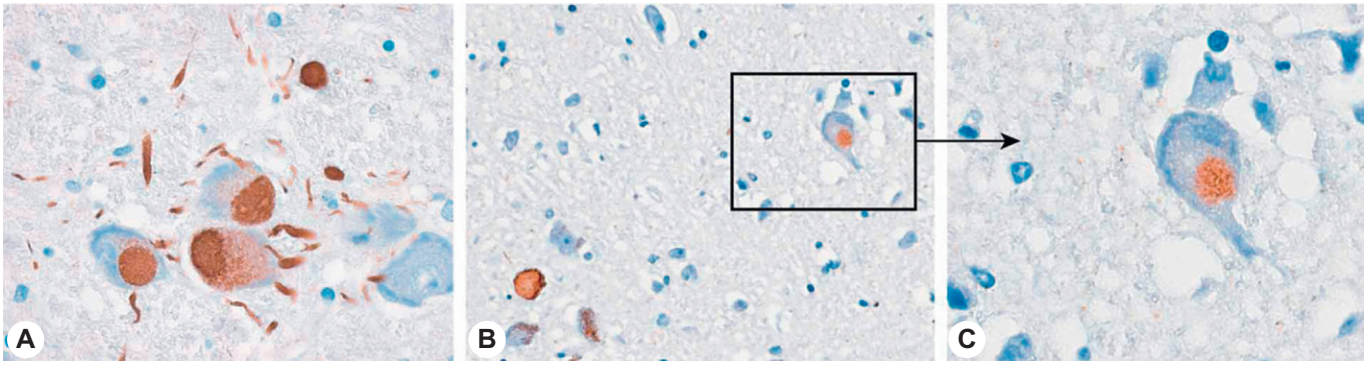


FIGURE 47-3 **Host-to-graft spreading of Lewy body pathology in Parkinson's disease.** The patient received a transplant of fetal human mesencephalic dopaminergic nerve cells into the putamen 16 years previously. Immunohistochemistry for α-synuclein visualizes Lewy bodies and Lewy neurites in **(A)** the host substantia nigra and **(B, C)** the transplant. Adapted from reference (Li et al., 2008).

Based on a model derived from solid-state nuclear magnetic resonance studies, the core of the α-synuclein filament comprises five β-strands reminiscent of a five-layered β-sandwich (Vilar et al., 2008). Only α-synuclein is associated with the filamentous inclusions of Lewy body diseases and α-synuclein-positive structures exceed those stained for ubiquitin, indicating that α-synuclein becomes ubiquitinated after assembly. Hyperphosphorylation at residue S129 is the major post-translational modification of filamentous α-synuclein (Fujiwara et al., 2002). G-protein–coupled receptor kinases, casein kinases and polo-like kinases phosphorylate S129. It remains to be shown unambiguously whether phosphorylation at S129 occurs before or after filament assembly.

Lewy body pathology is also the defining feature of several rarer diseases, such as Lewy body dysphagia and pure autonomic failure. In these diseases, Lewy bodies and Lewy neurites are largely limited to the enteric and peripheral nervous systems. In PD, Lewy body pathology is also present in the enteric and autonomic nervous systems. Incidental Lewy body disease is defined by the presence of small numbers of Lewy bodies and Lewy neurites in the absence of clinical symptoms. It is observed in 5–10% of individuals over the age of 60 and may represent a preclinical form of disease.

The development of α-synuclein pathology is not random

In most individuals, the first α-synuclein-positive structures in the brain form in the dorsal motor nucleus of the glossopharyngeal and vagus nerves, the intermediate reticular zone, the olfactory bulb and the anterior olfactory nucleus (Braak et al., 2003). The pathology ascends from vulnerable regions in the medulla oblongata to the pontine tegmentum, midbrain, basal forebrain and cerebral cortex.

α-Synuclein deposits may even form earlier in the enteric and peripheral nervous systems, suggesting that Lewy body diseases originate outside the central nervous system. The first deposits develop in the form of Lewy neurites, indicating that the filamentous assembly of α-synuclein in axons may precede assembly in cell bodies and dendrites. Incidental Lewy body disease may be at one end of the spectrum of Lewy body diseases, with DLB at the other end, and with Lewy body dysphagia, pure autonomic failure and PD in between.

This pattern of spreading of α-synuclein pathology raises the possibility that the disease process may initiate in a single cell, from where it may spread in a prion-like manner between synaptically connected neurons. In support, studies of the brains of PD patients who had received fetal mesencephalic nerve cell transplants 11–16 years earlier revealed the presence of Lewy bodies in the grafts (Figure 47-3) (Li et al., 2008; Kordower et al., 2008). These findings are consistent with a spread of seeds from the diseased host tissues to the grafts, followed by the nucleated assembly of α-synuclein. Experimental evidence for the intercellular transfer of α-synuclein and the seeding of aggregation has been obtained (Desplats et al., 2009). In the grafts, up to 5% of dopaminergic neurons contained Lewy bodies, similar to the proportion of Lewy body-bearing neurons in the substantia nigra from PD patients (Greffard et al., 2010). Nerve cells with Lewy bodies may die within six months of inclusion formation, with Lewy bodies and nerve cell death reaching a steady state. The Lewy body pathology that can be associated with AD, the parkinsonism-dementia complex of Guam and pantothenate kinase-associated neurodegeneration is not an invariable feature of these diseases. Nevertheless, understanding why α-synuclein pathology develops in a proportion of cases may shed light on the mechanisms operating in diseases defined by the presence of Lewy body pathology.

Other genes are implicated in Parkinson's disease

Mutations in the gene encoding the leucine-rich repeat kinase-2 (LRRK2) are associated with a substantial proportion of dominantly inherited PD (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). LRRK2 is a 2,527 amino acid protein with a leucine-rich repeat domain, a Ras-like small GTPase and a protein kinase domain. Pathogenic missense mutations are found in several domains. Most mutations give rise to clinical and pathological PD. However, some mutations cause pure nigral degeneration, whereas others result in the formation of neurofibrillary lesions in the absence of Lewy body pathology. Although LRRK2 and α-synuclein may function

in the same pathway, there is no evidence to suggest a direct interaction.

Genome-wide association studies (Satake et al., 2009; Simón-Sánchez et al., 2009), which identify genetic risk factors for idiopathic PD, have confirmed earlier work implicating polymorphic variants of *SNCA* and the H1 allele of *MAPT*. An association between PD and LRRK2 has been found, but it remains to be seen whether it can be accounted for by the known pathogenic mutations. Identification of the *HLA* locus as a risk factor for PD (Saiki et al., 2010) has suggested that repair mechanisms downstream of the primary insult may be important. The glucocerebrosidase gene (*GBA*), another susceptibility factor for PD, encodes an enzyme, which catalyzes the breakdown of glucosylceramide into ceramide and glucose in lysosomes (Sidransky et al., 2009). *GBA* is mutated in Gaucher's disease, which features the accumulation of glucosylceramide in lysosomes. The link with PD has been adduced from the occurrence of parkinsonism and Lewy body pathology in patients with Gaucher's disease and their relatives, and the identification of *GBA* mutations in PD. These findings indicate a connection between glycolipid metabolism and filament assembly of α-synuclein. *BST1*, which encodes a protein that generates cyclic ADP-ribose, a second messenger mobilizing calcium in the endoplasmic reticulum, is also associated with PD (Satake et al., 2009).

MULTIPLE SYSTEM ATROPHY

Glial cytoplasmic inclusions (Papp-Lantos inclusions), which consist of filamentous aggregates, are the defining neuropathological feature of multiple system atrophy (MSA). They are found mostly in the cytoplasm and, to a lesser extent, the nucleus of oligodendrocytes. Inclusions are also observed in the cytoplasm and nucleus of some nerve cells and in neuropil threads. The affected brain regions are mainly the substantia nigra, striatum, locus coeruleus, pontine nuclei, inferior olives, cerebellum and spinal cord. Typically, nerve cell loss and gliosis are observed. The formation of glial cytoplasmic inclusions may be the primary lesion that will eventually compromise nerve cell function and viability.

Glial cytoplasmic inclusions are immunoreactive for α-synuclein and their constituent filaments are labeled by α-synuclein antibodies (Spillantini et al., 1998). Assembled α-synuclein is phosphorylated at S129 and the number of α-synuclein-positive structures exceeds that stained for ubiquitin. Filament morphologies differ between MSA and Lewy body diseases, suggesting that distinct conformers of assembled α-synuclein can give rise to different neurodegenerative diseases. Sequence variation in *SCNA* is also a risk factor for multiple system atrophy (Al-Chalabi et al., 2009).

SYNTHETIC α-SYNUCLEIN FILAMENTS

Filaments made from recombinant monomeric human α-synuclein have the same morphological and ultrastructural characteristics as disease filaments (Figure 47-4). Assembly is a nucleation-dependent process that occurs through the amino-terminal repeats. The carboxy-terminal region, by contrast, is inhibitory. Under the conditions of these experiments, β- and γ-synucleins fail to assemble, consistent with their absence from disease filaments. Fibrillogenesis of human α-synuclein is dependent on β-strand contiguity and propensity, hydrophilicity and charge (Zibaee et al., 2007). Assembly assays lend themselves to scaling up for the identification of pharmacological modifiers of filament formation. Nontoxic, SDS-stable dimers and oligomers of α-synuclein form in the presence of compounds that inhibit filament formation (Masuda et al., 2006). Mutations E46K and A53T increase the rate of assembly of α-synuclein. The mechanism of action of the A30P mutation is less clear. It has been reported that it causes the accumulation of oligomeric, nonfibrillar α-synuclein, implying that this may be a toxic species. Mutation A30P reduces the binding of α-synuclein to rat brain vesicles, whereas mutation E46K increases lipid binding. Mutation A53T has no significant effect on lipid binding.

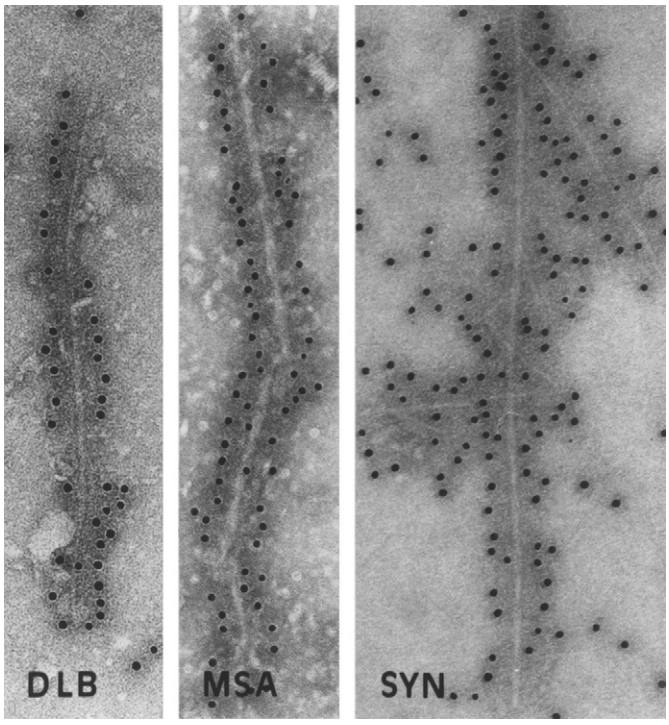


FIGURE 47-4 **Filaments extracted from the brains of patients with dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) or assembled from bacterially expressed monomeric human α-synuclein (SYN) were decorated by an anti-α-synuclein antibody.** The gold particles conjugated to the secondary antibody appear as black dots.

ANIMAL MODELS OF SYNUCLEINOPATHIES

Rodents and primates

Overexpression of wild-type α-synuclein inhibits evoked neurotransmitter release (Larsen et al., 2006). Overexpression

of α-synuclein has also been reported to inhibit macroautophagy, suggesting a link with Lewy body formation and neurodegeneration. Mice expressing human mutant α-synuclein in nerve cells or glial cells develop numerous α-synuclein-immunoreactive cell bodies and processes, with filament formation and nerve cell loss being less consistent features. One study has described the presence of α-synuclein filaments in brain and spinal cord of mice transgenic for human A53T α-synuclein (Giasson et al., 2002). The formation of inclusions correlated with the appearance of a movement disorder. In transgenic mice, overexpression of LRRK2 exacerbates α-synuclein pathology, with the *LRRK2* knockout reducing it (Lin et al., 2009). A major difference with PD is the absence of significant pathology and neurodegeneration in dopaminergic cells of the substantia nigra. This has been partially achieved following expression of carboxy-terminally truncated human α-synuclein (Tofaris et al., 2006). These mice also exhibited a reduction in the release of dopamine in the striatum. However, a transgenic mouse model that fully recapitulates the behavioral phenotype, neuropathology and pathophysiology of PD remains to be produced. A neurotoxin model of α-synuclein pathology has been developed in the rat through the chronic administration of the pesticide rotenone, a high-affinity inhibitor of mitochondrial complex I (Betarbet et al., 2000). Although the overall variability was substantial, some rats developed progressive degeneration of nigrostriatal neurons and Lewy body-like inclusions that were immunoreactive for α-synuclein and ubiquitin. They exhibited bradykinesia, postural instability and resting tremor. Inhibition of complex I was only partial, suggesting that reactive oxygen species can link mitochondrial dysfunction to α-synuclein aggregation. Intragastric infusion of rotenone has been shown to cause local accumulation of α-synuclein and its subsequent spreading to the brain (Pan-Montojo et al., 2010).

Adeno-associated and lentiviral vectors have been used to express human wild-type and mutant α-synuclein in rodent and primate substantia nigra. Lewy body-like inclusions formed and a significant proportion of nerve cells degenerated (Kirik et al., 2003). Lentiviral expression in the rat substantia nigra has shown that mutants of α-synuclein that form oligomers rather than filaments are more toxic than mutants that readily assemble into filaments (Winner et al., 2011), consistent with the view that oligomeric species of α-synuclein are the most toxic form.

Flies, worms and yeasts

Overexpression of human α-synuclein in *D. melanogaster* results in the formation of filamentous Lewy body-like inclusions, age-dependent loss of some dopaminergic neurons and locomotor defects (Feany & Bender, 2000). Chaperones modulate these effects. Overexpression of human α-synuclein in *C. elegans* also results in dopaminergic nerve cell loss and motor deficits. Human α-synuclein is toxic in yeast in a dose-dependent manner (Outeiro & Lindquist, 2003). Genome-wide screens have identified proteins involved in vesicle transport, lipid metabolism and protein degradation as modifiers of α-synuclein toxicity, indicating that lipid binding and vesicle transport are important for early toxic events. Aggregation of α-synuclein may be relevant, since β-synuclein and a non-aggregating form of α-synuclein were not toxic in yeast, despite their ability to bind lipids (Soper et al., 2008).

SYNUCLEINOPATHIES—OUTLOOK

A pathway leading from soluble α-synuclein to insoluble, filamentous α-synuclein is central to Lewy body diseases and MSA. The development of ever better experimental models for synucleinopathies makes it possible to discover the mechanisms that cause disease and to identify disease modifiers, which may result in the development of mechanism-based therapies. It remains to be seen if α-synuclein plays a role in the inherited forms of parkinsonism caused by loss-of-function mutations in *Parkin*, *DJ-1* and *PINK1*. The relevant proteins are required for the elimination of dysfunctional mitochondria (Ahlskog, 2009). A potential connection between Lewy body pathology and *PINK1*-linked parkinsonism has been described (Samaranch et al., 2009) (See chapters 41, 49).

MICROTUBULE-ASSOCIATED PROTEIN TAU

Six tau isoforms are expressed in adult human brain

The microtubule-binding protein tau is important for the assembly and stabilization of microtubules (Goedert & Spillantini, 2006). In nerve cells, tau is concentrated in axons, but in the human tauopathies it also accumulates in cell bodies and dendrites. Six isoforms are produced from a single *MAPT* gene on chromosome 17q21 by alternative mRNA splicing and differ from one another by the presence or absence of a 29- or 58-amino-acid insert in the amino-terminal half and by the inclusion, or not, of a 31-amino-acid repeat encoded by exon 10 in the carboxy-terminal half of the protein (Figure 47-5A). The exclusion of exon 10 leads to the production of three isoforms, each containing three repeats, and its inclusion to a further three isoforms, each containing four repeats. The repeats constitute the microtubule-binding region, with four-repeat tau being better at promoting microtubule assembly than tau with three repeats. In adult human brain, there are similar levels of three- and four-repeat isoforms. In developing human brain, only the shortest tau isoform (three repeats and no amino-terminal inserts) is expressed. In the peripheral nervous system, inclusion of exon 4A in the amino-terminal half results in the expression of high-molecular weight proteins called big tau. Although tau is expressed as multiple forms in vertebrates, isoform ratios are not conserved between species. For instance, tau isoforms with three, four or five repeats are expressed in adult chicken brain, whereas adult rodents express predominantly tau isoforms with four repeats.

Similar repeats are present in the otherwise unrelated high-molecular-weight microtubule-associated proteins MAP2 and MAP4. Repeat sequences are conserved throughout evolution, with the genomes of *C. elegans* and *D. melanogaster* each

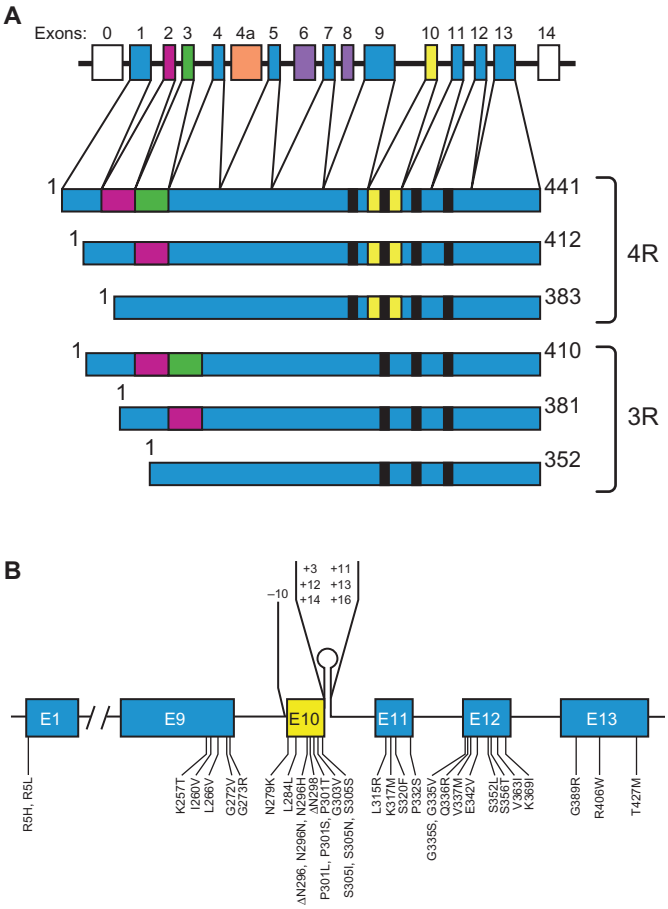


FIGURE 47-5 **(A), *MAPT* and the six tau isoforms expressed in adult human brain.** *MAPT* consists of 16 exons (E). Alternative mRNA splicing of E2 (red), E3 (green) and E10 (yellow) gives rise to the six tau isoforms (352-441 amino acids). The constitutively spliced exons (E1, E4, E5, E7, E9, E11, E12, E13) are indicated in blue. E0, which is part of the promoter, and E14 are non-coding (white). E6 and E8 (violet) are not transcribed in human brain. E4a (orange) is only expressed in the peripheral nervous system. Black bars indicate the microtubule-binding repeats of tau, with three isoforms having 4 repeats each (4R) and three isoforms having 3 repeats each (3R). The exons and introns are not drawn to scale. **(B)**, Mutations in *MAPT* in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17T). Thirty-six coding region mutations in exons (E) 1, 9, 10, 11, 12 and 13, and seven intronic mutations flanking E10 are shown.

encoding one protein with tau-like repeats. Inactivation of *MAPT* by homologous recombination results in mice that are largely normal (Harada et al., 1994). However, heterozygous microdeletions of the region of chromosome 17 that encompasses *MAPT* give rise to mental retardation in humans, consistent with a role for tau during brain development (See Box).

Tau is a phosphoprotein

Phosphorylation negatively regulates the ability of tau to interact with microtubules (Lindwall & Cole, 1984). Many phosphorylated sites are serine/threonine-prolines, 17 of which are found in the longest human brain tau isoform. Most of these sites flank the microtubule-binding repeats. Proline-directed protein kinases, such as glycogen synthase kinase-3 (GSK3), cyclin-dependent kinase-5 (CDK5), mitogen-activated protein (MAP) kinase and stress-activated protein (SAP) kinases, phosphorylate these sites *in vitro*. Lithium chloride reduces tau phosphorylation in rodent brain, indicating that GSK3 is a physiological tau kinase. Other sites in tau are phosphorylated *in vitro* by AGC kinases, calcium/calmodulin-dependent protein kinase II and members of the AMP-activated protein kinase (AMPK)-related kinase family. Some of these sites are located within the repeat region. Protein phosphatase 2A (PP2A) is the major phosphatase activity in brain towards tau phosphorylated by proline-directed and other kinases. Phosphorylation is developmentally regulated, such that in fetal brain, tau is phosphorylated more than in adult brain. An increase in tau phosphorylation in the early stages of tauopathy has been described in the brains of hibernating mammals, suggesting a possible link with hypometabolism (Stieler et al., 2011). In adult mouse brain, cold-water stress and starvation lead to the hyperphosphorylation of tau, with inhibition of PP2A activity rather than protein kinase activation being involved.

Human brain tau is also modified by O-GlcNAcylation, where N-acetyl-D-glucosamine becomes attached to the hydroxyl groups of serine/threonine residues. An inverse relationship between O-GlcNAcylation and phosphorylation of tau has been demonstrated (Lefebre et al., 2003). It follows that downregulation of O-GlcNAcylation could result in the hyperphosphorylation of tau.

TAU AND ALZHEIMER'S DISEASE

The paired helical filament is made of tau protein

Abundant neuritic plaques and neurofibrillary lesions define AD (see chapter 46). Plaques are extracellular deposits made of the fibrillar β-amyloid peptide. The paired helical filament (PHF) makes up the bulk of the intraneuronal neurofibrillary pathology, with the straight filament (SF) being a minority species. By the early 1990s, it was clear that tau protein is the major component of PHFs and SFs and that all six brain isoforms are present, each full-length and hyperphosphorylated (Goedert et al., 1992). The microtubule-binding repeat region of tau forms the core of the filament, with the amino- and carboxy-terminal regions forming a fuzzy coat around the filament. PHFs and SFs have a cross-β structure characteristic of amyloid fibres. Following assembly, tau becomes truncated at the amino-terminus, which is necessary for its subsequent ubiquitination. Following the demise of neurofibrillary tangle-bearing cells, the pathological material stays around in the form of so-called 'ghost tangles,' which consist mostly of the ubiquitinated repeat region of tau.

Filamentous tau is hyperphosphorylated

Hyperphosphorylation is an early event that appears to precede tau filament assembly. Much effort has gone into mapping phosphorylation sites and identifying candidate protein kinases and phosphatases (Hanger et al., 2009). For sites that

are phosphorylated in normal brain, a higher proportion of molecules are phosphorylated in filamentous tau. The latter is also phosphorylated at more serine, threonine and tyrosine residues than tau from normal adult brain. The mechanisms underlying filament formation in neurons are still unclear, but hyperphosphorylation may disengage tau from microtubules, thereby increasing the pool of unbound tau, which may be more resistant to degradation and more prone to aggregation than microtubule-bound tau. It suggests that an increased ability of pathological tau to interact with microtubules may be beneficial. This could be achieved through the inhibition of protein kinases and/or activation of protein phosphatases. Osmolytes such as trimethylamine N-oxide (TMAO) and betaine can restore the ability of phosphorylated tau to interact with microtubules, probably through a conformational change in tubulin and/or tau.

The development of tau pathology is not random

Tau pathology follows a stereotyped pattern with regard to affected nerve cell types, cellular layers and brain regions, with little inter-individual variation. This has been used to define six neuropathological stages of AD (Braak & Braak, 1991; Braak & Del Tredici, 2011). Hyperphosphorylated tau first appears in axons of noradrenergic nerve cells of the ceruleus/subceruleus complex, followed by their cell bodies and dendrites. Staining of pyramidal cells in the transentorhinal cortex is observed next, some of which are also silver-positive, indicative of the assembly of tau into filaments. These changes define stage I. Stage II shows a more severe involvement of this region, as well as a mild involvement of the entorhinal cortex. Patients with these pathologies are cognitively unimpaired. Mild impairment of cognitive function becomes apparent in stages III and IV. Stage III is characterized by severe neurofibrillary lesions in the pre-α layer of entorhinal and transentorhinal regions. During stages III and IV, mild changes are also present in layer I of Ammon's horn of the hippocampus and in a number of subcortical nuclei, such as the basal forebrain magnocellular nuclei and the anterodorsal thalamic nucleus. The major feature of stages V and VI is the massive development of neurofibrillary pathology in isocortical association areas. They meet the criteria for a neuropathological diagnosis of AD and are found in patients who are severely demented. The stereotyped nature of the spatial and temporal development of neurofibrillary pathology contrasts with the development of Aβ deposits. They show a density and distribution that are subject to great individual variation.

OTHER TAUOPATHIES

Other taupathies include progressive supranuclear palsy, corticobasal degeneration and Pick's disease

These diseases exhibit abundant filamentous tau inclusions, in the absence of extracellular amyloid deposits. As in AD, it is the anatomical distribution of tau pathology that determines the nature of the clinical syndrome (Goedert & Spillantini, 2006). Progressive supranuclear palsy (PSP) leads to vertical supranuclear gaze palsy, postural instability and cognitive impairment. It is clinically and pathologically heterogeneous, with pathology being most prominent in basal ganglia, diencephalon, brainstem, cerebellum and cerebral cortex. Like PSP, corticobasal degeneration (CBD) is an atypical parkinsonian disorder. It leads to depigmentation of the substantia nigra and asymmetric frontoparietal atrophy. Pick's disease is characterized by frontotemporal lobar and limbic atrophy. Other tauopathies include Guam parkinsonism–dementia complex, argyrophilic grain disease, white matter tauopathy with globular glial inclusions and Guadeloupean parkinsonism. Tau filament morphologies differ between diseases, with hyperphosphorylated sites being mostly shared. The phosphorylated sites are similar to those of AD. However, the tau isoform composition in the filaments can vary. Like AD and other diseases with extracellular deposits, Guam parkinsonism–dementia complex is characterized by the presence of all six tau isoforms. In Pick's disease filaments, tau isoforms with three repeats predominate. By contrast, filaments from PSP, CBD, argyrophilic grain disease, white matter tauopathy with globular glial inclusions and Guadeloupean parkinsonism consist of tau isoforms with four repeats. This correlates with the presence of tau inclusions, not only in nerve cells, but also in glial cells, chiefly astrocytes and oligodendrocytes. Together with the stereotyped appearance of tau inclusions in the disease process of AD, these findings suggest that self-propagating conformers of tau may exist, akin to the prion strains arising from the conformational variability of PrP^{Sc} (Colby & Prusiner, 2011) (See Ch. 50). In support, experimental evidence for the transmission, spreading and intercellular transfer of tau aggregates has been adduced (Frost et al., 2009; Clavaguera et al., 2009).

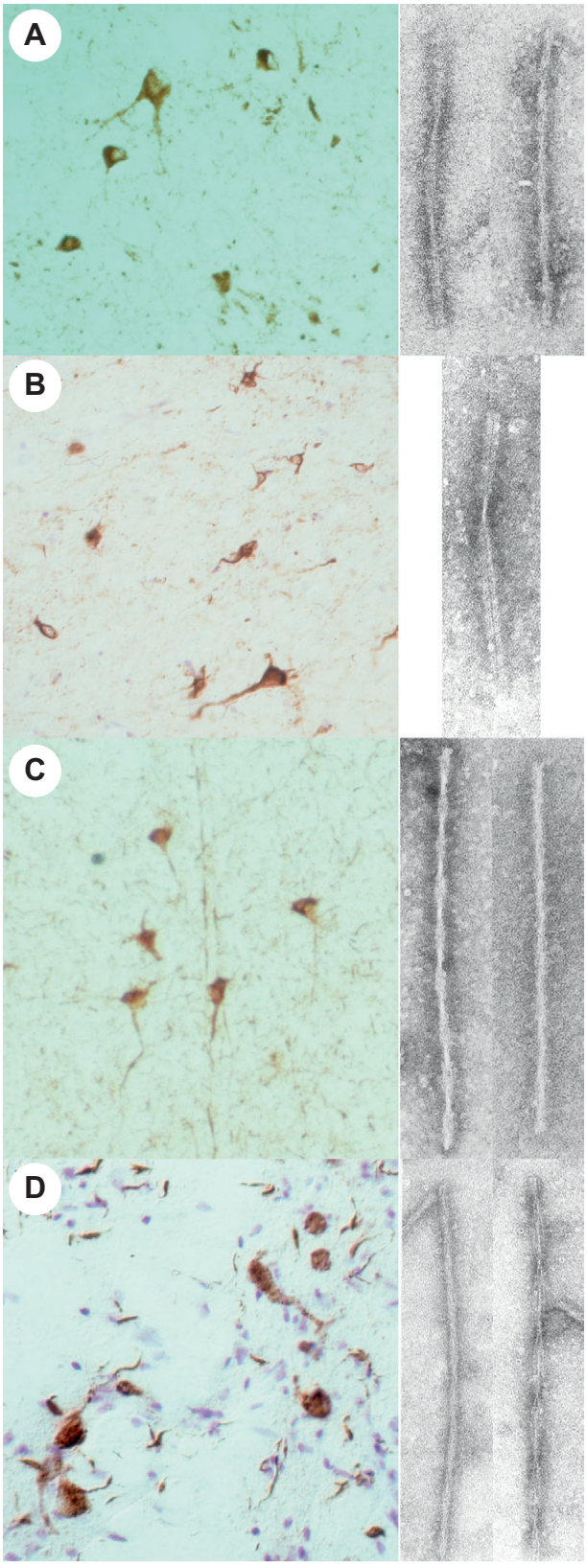
MAPT MUTATIONS CAUSING TAUOPATHY

FTD is characterized by atrophy of the frontal and temporal lobes of the cerebral cortex, with additional subcortical changes

In 1994, an autosomal dominant inherited form of FTD with parkinsonism was linked to chromosome 17q21.2 (Wilhelmsen et al., 1994). Subsequently, other forms of disease were linked to this region, resulting in the denomination 'frontotemporal dementia and parkinsonism linked to chromosome 17' (FTDP-17). Mutations in *MAPT* (Poorkaj et al., 1998; Hutton et al., 1998; Spillantini et al., 1998) or the progranulin gene (*GRN*) (Baker et al., 2006; Cruts et al., 2006) cause FTDP-17 (Figure 47-5B). Over 40 mutations in *MAPT* have been identified in FTDP-17T. They give rise to a filamentous tau pathology (Figure 47-6).

MAPT mutations are exonic or intronic

Missense, deletion and silent mutations are present in the coding region, with intronic mutations being located



close to the splice sites of the introns flanking exon 10 of *MAPT* (Goedert & Spillantini, 2006). Functionally, they fall into two largely non-overlapping categories—those whose primary effect is at the protein level, and those influencing the alternative splicing of tau pre-mRNA. Most missense mutations reduce the ability of tau to interact with microtubules, with some mutations also promoting aggregation. They lead to the formation of nerve cell inclusions that consist of filaments made of all six brain tau isoforms. Mutations P301L and P301S in exon 10 are exceptions, since they lead to the formation of neuronal and glial inclusions made of tau isoforms with four repeats. This may also be true of mutation P301T.

Intronic mutations and most coding region mutations in exon 10 increase the splicing of exon 10, changing the ratio of three- to four-repeat tau isoforms, resulting in the relative overproduction of four-repeat tau. Filaments made of four-repeat tau are found in both nerve cells and glial cells. Approximately 40% of known mutations have their primary effect at the RNA level. Mutations in exon 10 affect splicing enhancer or silencer sequences, with mutations in the intron following exon 10 destabilizing a stem–loop structure. To a significant degree, FTDP-17T is a disease of the alternative mRNA splicing of exon 10 of *MAPT*, with a physiological ratio of tau isoforms being essential for preventing neurodegeneration and dementia.

RELEVANCE FOR OTHER TAUOPATHIES

The study of FTDP-17T has revealed that dysfunction or misregulation of tau protein can cause neurodegeneration and dementia (Ghetti et al., 2011). It follows that tau is also of central importance for the pathogenesis of AD, PSP, CBD and Pick's disease. Nine missense mutations in *MAPT* give rise to a clinical and neuropathological phenotype like that of Pick's disease. The finding that overproduction of four-repeat tau causes disease and leads to the assembly of four-repeat tau in nerve cells and glial cells may shed light on the pathogenesis of PSP and CBD. Both diseases are characterized by neuronal and glial deposits made of four-repeat tau. Haplotypes H1 and H2 characterize *MAPT* in populations of European descent and result from a 900 kb inversion/non-inversion (H1/H2) polymorphism (Conrad et al., 1997; Stefansson et al., 2005). Inheritance of

FIGURE 47-6 **Pathologies of FTDP-17T, as revealed by staining for hyperphosphorylated tau protein and the morphologies of isolated tau filaments. (A)**, Mutation P301L in exon 10 gives rise to a neuronal and glial tau pathology. Filaments consist of a majority of narrow twisted ribbons (left) and a minority of rope-like filaments (right). **(B)**, Mutations in the intron following exon 10 give rise to a neuronal and glial tau pathology. Filaments consist of wide twisted ribbons. **(C)**, Mutation V337M in exon 12 gives rise to a neuronal tau pathology. Filaments consist of paired helical filaments (left) and straight filaments (right), like the tau filaments of Alzheimer's disease. **(D)**, Mutation G389R in exon 13 gives rise to a neuronal tau pathology. Filaments consist of a majority of straight filaments (left) and a minority of twisted filaments (right). The tau pathology resembles that of Pick's disease.

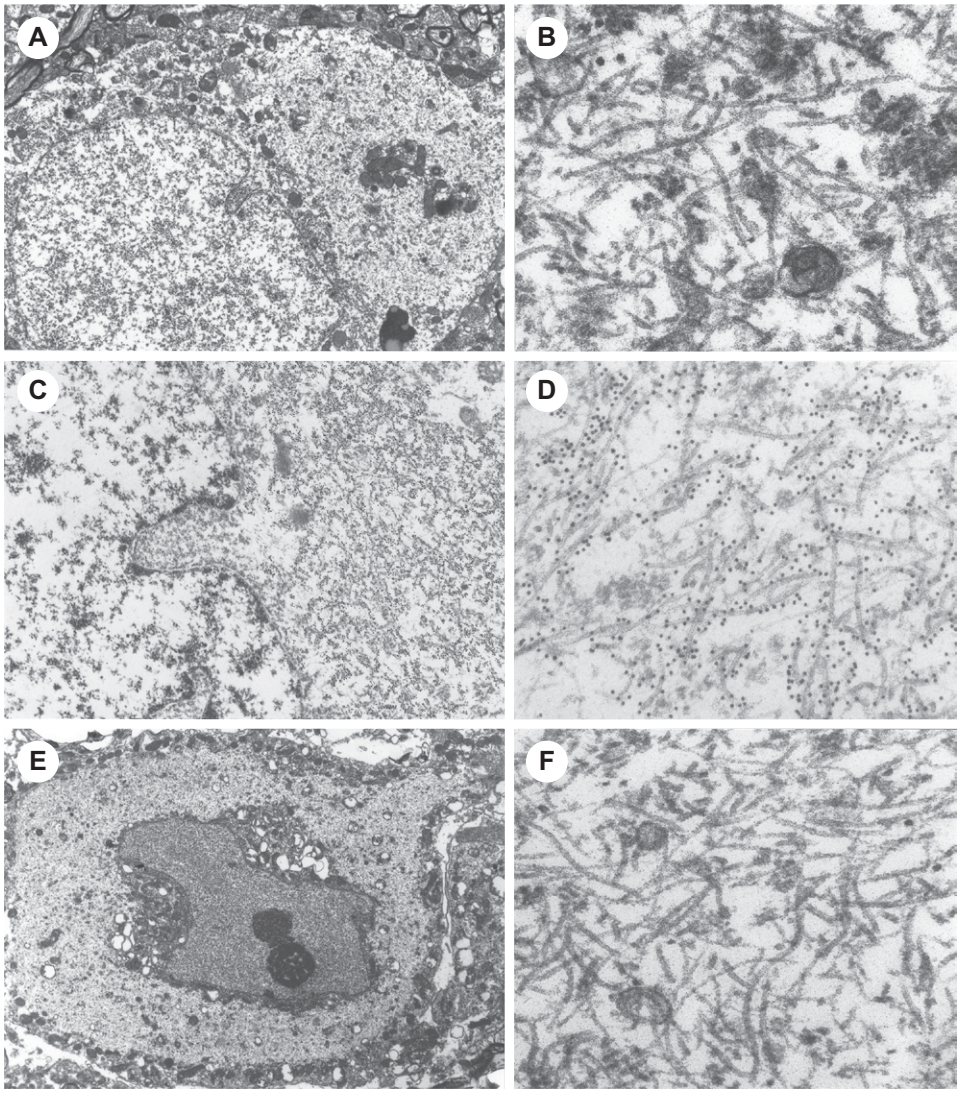


FIGURE 47-7 **Tau filaments in brain and spinal cord from mice transgenic for human mutant P301S tau protein. (A, B)** Cerebral cortex. **(C, D)** Brainstem. **(E, F)** Spinal cord. **(B, D, F)**: Higher magnification of parts of the cytoplasmic regions from **(A, C, E)**. The electron micrographs in **(C)** and **(D)** show immunogold labelling of filaments using the phosphorylation-dependent anti-tau antibody AT8.

the H1 haplotype is a risk factor for PSP and CBD. This was confirmed in a genome-wide association study, which has also implicated proteins involved in vesicle trafficking, the unfolded protein response and the innate immune system (Höglinger et al., 2011). The increased risk of PSP and CBD conferred by the H1 haplotype appears to promote *MAPT* transcription and incorporation of exon 10, resulting in increased levels of four-repeat tau.

SYNTHETIC TAU FILAMENTS

Tau filaments can be assembled *in vitro* from the microtubule-binding repeat region, confirming that it determines filament morphology. Truncation of tau has been shown to promote filament assembly in cells (Wang et al., 2007), but it remains to be seen whether it is also necessary for filament formation in human brain. The most soluble filaments from diseased human brain consist of full-length tau, and extensive truncation occurs after assembly (Goedert & Spillantini, 2006). Sulphated glycosaminoglycans, RNA and fatty acids induce the bulk assembly of full-length tau into filaments. Like brain filaments, synthetic tau filaments can be decorated by antibodies directed against the amino- and carboxy-termini of tau, but not by an antibody against the microtubule-binding repeats. Short amino acid sequences in the second (^{275}VQIINK^{280}) and third (^{306}VQIVYK^{311}) microtubule-binding repeats are essential for the heparin-induced assembly of tau into filaments. Nontoxic, SDS-stable oligomers of tau form in the presence of compounds that inhibit filament formation (Masuda et al., 2006). The relevance of phosphorylation for the *in vitro* assembly of full-length tau is not clear, with reports ranging from no influence to stimulatory and inhibitory effects.

ANIMAL MODELS OF HUMAN TAUOPATHIES

Rodents and fish

Mice expressing human mutant tau in nerve cells or glial cells develop filamentous deposits made of hyperphosphorylated tau

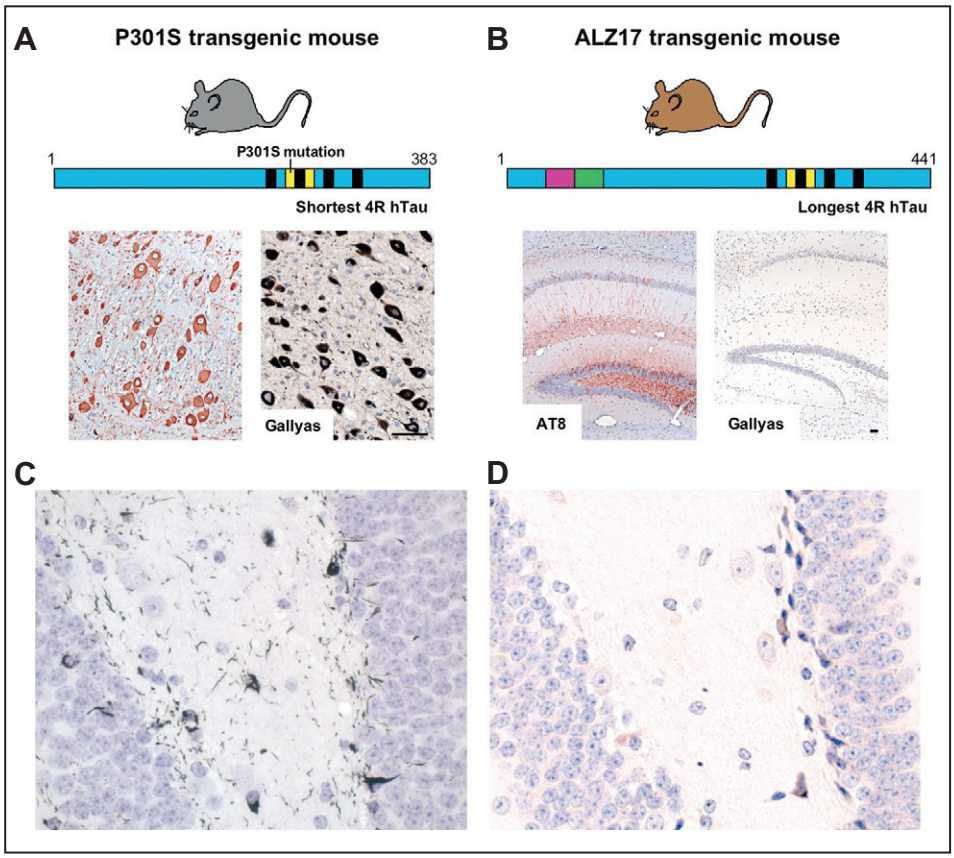


FIGURE 47-8 **Induction of filamentous tau pathology in the brain of transgenic ALZ17 mice expressing human wild-type tau following the injection of brain extract from mice transgenic for human mutant P301S tau. (A)**, Mice expressing the 383 amino acid four-repeat tau isoform of human tau (4R hTau) with the P301S mutation under the control of the murine Thy1 promoter develop abundant Gallyas-Braak silver-positive filamentous tau inclusions and widespread nerve cell loss, including in the brainstem, the brain region used for preparation of the extract injected in **(C)** and **(D)**. The silver-positive tau inclusions are immunoreactive with antibody AT8, a marker for hyperphosphorylated tau. In humans, mutation P301S causes an aggressive form of FTDP-17T. **(B)**, Mice expressing the 441 amino acid 4R hTau isoform under the control of the murine Thy1 promoter (line ALZ17) do not develop Gallyas-Braak silver-positive inclusions (right inset) or nerve cell loss, even though human tau is hyperphosphorylated at the AT8 epitope (left inset), as shown for the hippocampus. **(C)**, The injection of brain extract from P301S tau transgenic mice into the hippocampus and the cerebral cortex of ALZ17 mice induces the formation of Gallyas-Braak silver-positive inclusions made of filamentous, hyperphosphorylated wild-type human tau. The hippocampal dentate gyrus from an ALZ17 mouse is shown 15 months after the injection of brainstem extract from a 6-month-old P301S tau mouse. Silver-positive neurofibrillary tangles, neuropil threads and oligodendroglial coiled bodies are in evidence. **(D)**, Injection of the same extract as in **(C)**, but immunodepleted of tau, shows no Gallyas-Braak silver-positive inclusions 15 months later.

protein and neurodegeneration (Figure 47-7) (Lewis et al., 2000; Allen et al., 2002).

Hyperphosphorylation precedes filament assembly and increased phosphorylation of soluble tau results in increased filament formation, suggesting that phosphorylation of tau can drive filament assembly. Extracellular deposits made of Aβ or Danish amyloid promote filament formation of human mutant tau, demonstrating that extracellular amyloid can drive intraneuronal tau pathology (Coomaraswamy et al., 2010). Phosphorylation of tau by GSK3β and AMP-activated protein kinase are potential mechanisms. Tau protein is required for Aβ toxicity in experimental models (Roberson et al., 2007). The absence of Aβ toxicity in mice lacking *MAPT* may result from reduced excitotoxicity because of decreased dendritic localization of the tyrosine kinase Fyn, resulting in hypophosphorylation of the NMDA receptor and a reduced interaction with postsynaptic density protein-95 (Ittner et al., 2010). Filamentous tau deposits also formed in a mouse line expressing all six wild-type human brain tau isoforms in the absence of mouse tau (Andorfer et al., 2003). Surprisingly, haploinsufficiency of p73, a member of the p53 protein family, has been reported to be associated with the formation of tau aggregates in nerve cells and to potentiate Aβ toxicity, possibly through the activation of SAP kinases (Wetzel. et al., 2008). In rodent brain, adeno-associated virus-mediated expression of mutant human tau led to the formation of filamentous deposits of hyperphosphorylated tau protein (Klein et al., 2010). Although tau inclusions form in many neurodegenerative diseases, their role in disease pathogenesis remains a subject for debate. Studies using transgenic mice overexpressing human mutant tau in a conditional manner have reported a dissociation between tangle formation and nerve cell death (Santacruz et al., 2005). It appears that soluble hyperphosphorylated tau contributes to nerve cell

TAU PROTEIN AS A SCAFFOLD FOR SIGNALING MOLECULES

Scott T. Brady

The best-known function of tau protein is as a microtubule-associated protein thought to regulate microtubule dynamics in the neuron, but the tau gene products contain many sequences that are not thought to play a role in microtubule binding. Many of the alternative splice forms involve regions of the molecule outside the microtubule interacting core, raising questions of a functional role for these domains. Several lines of evidence suggest that tau may serve to recruit specific signaling molecules to the microtubule cytoskeleton, perhaps acting as a scaffold to organize signaling pathways. The large number of phosphorylation sites throughout the tau sequence may reflect changes in this scaffold function as well as playing a role in regulation of tau binding to microtubules. The appeal of this idea has grown as the importance of scaffold proteins in organizing kinases and phosphatases in cells has become apparent for many pathways, including PKA, PKC, GSK3, JNK and others (Vondriska et al., 2004).

Phosphatases were among the first phosphotransferases reported to bind tau. Protein phosphatases PP1, PP2A and calcineurin (PP2B) are all reported to interact with microtubules via an interaction with tau protein. Various kinase activities have similarly been reported to interact with microtubules through tau, including cdk5, GSK3β, Fyn and PI3 kinase.

Given the dramatic alterations in kinase activities seen in Alzheimer's disease and other tauopathies (Crews & Masliah, 2010), a consideration of how tau interactions with these various kinases and phosphatases differs in native and pathogenic conformations might illuminate the altered signaling pathways in these diseases. The demonstration that the central proline rich region of tau allows Fyn to dock with tau and modify Y18 (Lee, 2005) provides one example, because the proline-rich region is thought to be buried in the filament. Similarly, a recent study examined the differential effects of soluble and filamentous tau on fast axonal transport. Soluble, monomeric tau at physiological concentrations had no effect on axonal transport, but tau filaments at the same concentrations selectively inhibited kinesin-based axonal transport. Pharmacological experiments indicated that tau filament effects on FAT were mediated by PP1 and GSK3β (Lapointe et al., 2009). Remarkably, deletion of a conserved 18-amino-acid sequence at the tau N-terminus abolished this effect, suggesting that the tau N-terminus may have a role in regulating PP1/GSK3β in the axon. Studies like these suggest that tau may play an important role in regulating signal pathways associated with microtubules, including ones critical in Alzheimer's and other tauopathies.

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dysfunction prior to assembly into filaments. A reduction of tau kinase activity, an increase of tau O-GlcNAcylation and an increase of tau phosphatase activity may therefore be of therapeutic benefit. Promising results have been obtained in mouse models of human tauopathy (Le Corre et al., 2006; Van Eersel et al., 2010).

Injection of sonicated brain extract from mice with abundant tau inclusions into the cerebral cortex and hippocampus of transgenic mice lacking inclusions induces the assembly of human wild-type tau into filaments and leads to the spreading of pathology from the injection sites to neighbouring brain regions (Figure 47-8) (Clavaguera et al., 2009). Injection of brain extract immunodepleted of tau or divided into soluble and insoluble fractions shows that insoluble tau induces aggregation, in the absence of obvious signs of neurodegeneration. Parallel work has demonstrated the transfer of aggregated tau between transfected non-neuronal cells (Frost et al., 2009). It thus appears that the tau species that are responsible for transmission and toxicity are not identical.

Use of the Gal4-UAS system in zebrafish has made it possible to produce a tauopathy model with a larval phenotype (Paquet et al., 2009). Expression of human mutant tau leads to misfolding and hyperphosphorylation of tau in motor neurones, as well as nerve cell loss. Argyrophilic inclusions are observed at later stages.

Flies, worms and yeasts

Expression of wild-type and mutant human tau in nerve cells of *D. melanogaster* and *C. elegans* leads to the loss of nerve cells and a reduced lifespan, in the apparent absence of tau filaments (Wittmann et al., 2001; Kraemer et al., 2003). However, abundant filaments form upon tau expression in fly glial cells. In genetic modifier screens, increasing kinase activity enhances tau toxicity, with an increase in phosphatase activity being beneficial (Feany 2010). Activation of antioxidant defenses is also beneficial. Oxidative stress has been linked to abnormal cell cycle activation, which is believed to result in neurodegeneration. Cell cycle activation in nerve cells of *Drosophila* is mediated through the target of rapamycin (TOR) pathway. Rapamycin, an inhibitor of TOR, reduces tau toxicity in *Drosophila*. It remains to be seen if this is related to reduced cell cycle activation or reflects induction of autophagy. In

contrast to what has been described in FTDP-17T and mouse models thereof, tau-induced neurodegeneration involves programmed cell death. As in mice, the neurotoxicity of tau in *Drosophila* is enhanced upon co-expression of Aβ, with oxidative stress, phosphorylation of S262/S356 and activation of DNA repair pathways being involved. In *C. elegans*, loss of a single gene, *sut-2* (suppressor of tau pathology-2), eliminates the toxic effects of human mutant tau, possibly via an increase in autophagic clearance (Guthrie et al., 2009). Only a few studies have investigated the expression of tau in yeasts. In *S. cerevisiae*, human tau is hyperphosphorylated and aggregates to some extent, but without binding to microtubules or inhibiting growth (Bharadwaj et al., 2010).

TAUOPATHIES—OUTLOOK

A neurodegenerative pathway leading from soluble to insoluble, filamentous tau is central to the neurodegenerative process in the human tauopathies. The availability of animal models exhibiting the essential molecular and cellular features of the human diseases has opened the way to a detailed understanding of the neurodegenerative process and the identification of genetic and pharmacological modifiers. Besides hyperphosphorylation and aggregation, clearance of tau, immunotherapy, cell replacement and microtubule stabilization are being pursued as potential therapies.

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