

Two to Tangle: The Story of Tau and α -Synuclein

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Summary

Recent research of neurodegenerative diseases has called attention to abnormal protein aggregates found in brain lesions. Alzheimer's disease (AD) and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) are part of a collective group of neurodegenerative diseases termed tauopathies and are characterized by abnormal filamentous tau inclusions. A_β amyloid plaques and neurofibrillary tangles containing hyperphosphorylated tau are invariant features of AD. The role of tau in AD pathogenesis is controversial. However, multiple tau mutations discovered in FTDP-17 add significance to tau's role in neurodegeneration. To study AD, our lab has identified different sites that become abnormally phosphorylated in adult tau and the potential role for dysfunctional phosphatases. To model FTDP-17, we and other labs have created transgenic mice that either overexpress wild type tau or express mutant tau. These mice develop clear neurodegenerative phenotypes. Analogous to tau aggregates, cytoplasmic inclusions known as Lewy bodies are characteristic of Parkinson's disease (PD). α -synuclein has been linked to both familial and sporadic PD. Following this discovery, our lab has demonstrated that aggregated α -synuclein appears in Lewy bodies of human patients. Transgenic mice and *Drosophila* models expressing α -synuclein are being used to identify suppressors and enhancers of disease. Investigating the role of tau and α -synuclein in neurodegenerative diseases can aid in the development of effective treatments.

Introduction

A common theme among many neurodegenerative disorders is the accumulation of abnormal proteins in the brain. Tauopathies and synucleinopathies are collectively grouped diseases involving protein aggregates of abnormal tau or α -synuclein, respectively. Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), Alzheimer's disease (AD), and Parkinson's disease (PD) belong to the above groups of diseases, though they display diverse phenotypes.

FTDP-17 and AD are adult-onset neurodegenerative dementias characterized by memory deficits and the progressive loss of cognitive abilities¹. The clinical symptoms of FTDP-17 also include behavioral disturbances, muscle rigidity, body tremor, bradykinesia, and amyotrophy (muscle weakness). AD symptoms relate more to deficits in problem solving, language, judgment, and behavior¹. Whereas FTDP-17 causes atrophy in the frontal neocortex, AD primarily affects neurons in the temporal lobe, hippocampus, and the parietal association cortices¹. Neurofibrillary tangles containing hyperphosphorylated tau, a microtubule-associated protein, are distinct features of both FTDP-17 and sporadic and familial AD^{1, 2} (Figure 1).

Along with AD, PD is also a common neurodegenerative disorder in the aging population. Symptoms of PD include resting tremor, rigidity, bradykinesia, gait disturbance, and postural instability³. Pathologically, PD is accompanied by a loss of dopaminergic neurons in the substantia nigra and by the presence of Lewy bodies (Figure 1). Both sporadic and familial PD involve misfolding of the α -synuclein protein (Figure 1). Sporadic PD, though less understood, is more widespread than familial PD. Oxidative stress and mitochondrial dysfunction are thought to play important roles in sporadic PD^{3, 4}. The familial form, however, allows the development of transgenic models and offers insights into the role of α -synuclein and the pathogenesis of PD³.

Twists and Tangles

The prominent features of AD are extracellular amyloid plaques and intracellular neurofibrillary tangles containing paired helical filaments (PHF)^{1, 5}. Amyloid plaques are primarily composed of amyloid- β -peptides (A_β), which are produced by cleavage of an amyloid precursor protein (APP)¹. Mutations in APP lead to overproduction of insoluble A_β peptide and its deposition in the neuritic plaques^{1, 2}. Although hyperphosphorylated tau was identified in neurofibrillary tangles (Figure 1), amyloid plaques were believed to be the dominant cause of neurodegeneration in AD. However, since tau inclusions were also seen in FTDP-17 patients, tau became a candidate gene for FTDP-17 pathology⁶. The discovery of several tau gene mutations added further significance to tau, indicating that abnormal tau might be a primary cause of neurodegeneration⁷.

The protein tau itself is found abundantly in axons of nerve cells and to some extent in glial cells. It binds to microtubules, stabilizes them, and facilitates transport of vesicles¹. Six isoforms of the tau protein are present in the adult human brain^{1, 2}. All six isoforms are produced by alternative splicing of the tau gene, and they differ by amino acid length and the number of tandem repeats (3R or 4R) of 31 or 32 amino acids¹. The microtubule binding domains are located on the tandem repeats². Whereas the adult brain contains all six isoforms of tau, the fetal brain only expresses the shortest tau isoform². Moreover, the hyperphosphorylation of tau is also developmentally regulated. Fetal tau is found to be more highly phosphorylated in immature brain than in adult brain. The level of phosphorylation for all six isoforms decreases with age².

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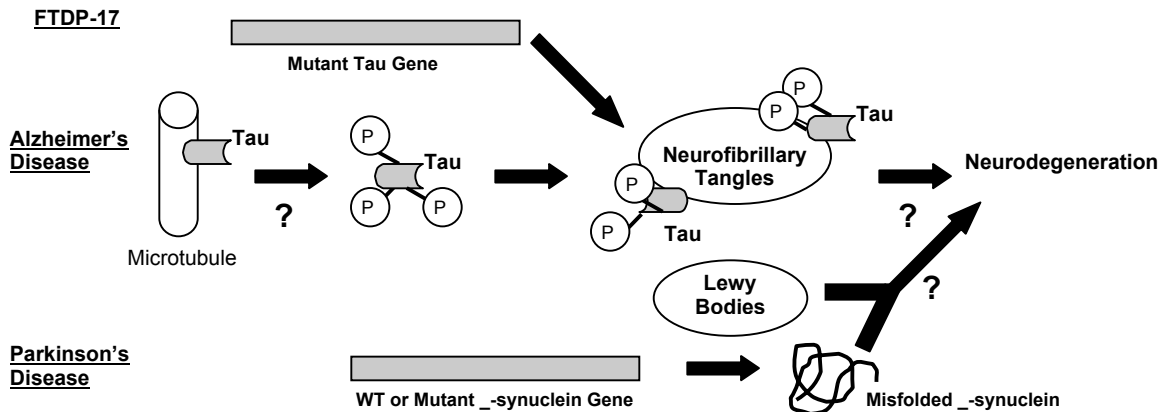


Figure 1. Role of tau and α -synuclein in neurodegenerative diseases

In FTDP-17, mutations in tau have been linked to the presence of neurofibrillary tangles containing hyperphosphorylated tau. The role of tau in pathogenesis of Alzheimer's disease is uncertain, but hyperphosphorylated tau has been identified in neurofibrillary tangles. In Parkinson's disease, mutations in α -synuclein have been identified, but how misfolded α -synuclein or Lewy bodies lead to neurodegeneration is unclear.

Mutations of tau in FTDP-17 are either missense, silent, and deletion mutations in the exons or intronic mutations^{7, 8, 9}. Intronic mutations near exon 10 (E10) of the tau gene increase the ratio of 4R-tau to 3R-tau, which is ~1 in the normal adult brain^{7, 8}. Studies suggest that mutations in tau destabilize an RNA stem-loop structure that follows E10, leading to an increased encoding of E10 and an increased proportion of 4R-tau^{7, 8}. In FTDP-17, hyperphosphorylated tau forms into twisted filaments that are different from PHF tau of AD⁵.

Following Mutant Tau

Soon after tau gene mutations were reported, our lab pursued investigation of the pathological mechanisms that cause FTDP-17. One of our studies demonstrates that splicing of E10 involves three different regulatory elements¹⁰. Missense tau mutations can act on an exon splicing enhancer within E10, and thereby increase the inclusion or exclusion of E10 in tau transcripts. Silent tau mutations can also increase inclusion of E10 by destroying the exon splicing silencer element within E10. Lastly, intronic mutations in intronic sequences flanking the 5' splice site of E10 enhance encoding of E10¹⁰. Since the tau gene is regulated by several elements, tau mutations can cause FTDP-17 through multiple mechanisms.

Tau Tangles Shy Away from Microtubules

Our lab has also analyzed the interaction between mutant tau and microtubules. Bacterial cells expressing recombinant tau protein with missense mutations show reduced binding of tau to microtubules¹⁰. Furthermore, the ability of tau to promote microtubule assembly is also reduced¹⁰.

A similar study has also been performed on Chinese hamster ovary (CHO) cells. The resulting pathological phenotypes included formation of insoluble tau aggregates and decreased microtubule binding¹¹. Both of these studies find that decreased levels of microtubule binding correlate with increased amounts of insoluble, unbound tau in the cytoplasm, which could eventually lead to hyperphosphorylation and aggregation of tau^{10, 11}.

Of Mice and Tau

In order to model FTDP-17, we and other labs have created transgenic mouse models that either

overexpress wild type tau or express mutant tau. Transgenic mice overexpressing the shortest tau isoform or the longest tau isoform developed accumulations of insoluble, hyperphosphorylated tau. Furthermore, these mice showed reduced fast axonal transport, axonal degeneration, diminished microtubules, and motor weakness^{12, 13}. Since these mice developed neuropathological symptoms by overexpression of normal tau, these studies also support the role of tau in AD pathogenesis.

Other labs have created transgenic mice expressing the common FTDP-17 mutation P301L. Similar to human pathology, these mice showed formation of neurofibrillary tangles and neuronal lesions^{14, 15}. Behavioral and motor deficits characteristic of FTDP-17 were also prominent in these transgenic mice¹⁴.

Tau Tangles in the AD Arena

Since PHFs in AD are composed of hyperphosphorylated forms of tau¹⁶, our lab set out to identify the different sites on normal adult tau that become abnormally phosphorylated. Most of these sites are serine (Ser) or threonine (Thr) residues. In addition, some of these sites, i.e. Ser262 and Ser396, are located near microtubule-binding domains^{17, 18, 19}.

Our lab proceeded to study whether these abnormally phosphorylated sites might be the result of inactivated protein phosphatases²⁰⁻²². Since the phosphorylation levels of tau isoforms decrease with age in normal adult brain, activation of protein phosphatases is one mechanism that might contribute to dephosphorylation. Protein phosphatases 2A (PP2A) and 2B (PP2B) were shown to dephosphorylate tau in developing rat brains²⁰. We have also demonstrated that in human postmitotic neuron-like cells (NT2N), disrupting the microtubule network results in site-specific dephosphorylation of tau by PP2A²¹. When NT2N cells are treated with inhibitors of PP2A, results indicate increased tau phosphorylation, decreased microtubule binding, destruction of selective microtubules, and axon degeneration²².

Following this series of studies, we next proceeded to compare mRNA distribution of PP2A in control and AD hippocampus. The level of PP2A mRNA was significantly reduced in AD hippocampus²³. Our data indicates that protein phosphatases may play

an important role in regulating the phosphorylation state of tau, and their activity is decreased in AD neurons.

Along with inactivation of phosphatases, activation of kinases can also be responsible for abnormal tau phosphorylation. One lab has shown that increased activity of cyclin-dependent kinase 5 (cdk5) also increases levels of tau phosphorylation in several cell lines²⁴. Brain proline-directed kinases (BPK) are microtubule-associated proteins that have also been shown to phosphorylate tau. Furthermore, BPK phosphorylates sites that are found abnormally phosphorylated in PHF tau²⁵.

Enhanced Tau Tangles

As previously described, transgenic mouse models overexpressing the shortest tau isoforms developed neurofibrillary tangles¹². Moreover, similar to AD patients, these tangles contained neurofilament (NF) subunits¹². Our lab extended these findings by designing transgenic mice that lacked neurofilaments²⁶. Thus, transgenic mice overexpressing the shortest tau isoform were crossbred with knock-out mice lacking neurofilaments. As a result, tau aggregation was delayed, weight loss was reduced, viability was increased, and behavioral phenotypes were improved when compared to transgenic mice with neurofilaments²⁶. Therefore, we found that neurofilaments may play a malevolent role in AD pathogenesis by facilitating aggregation of tau.

Plaques and Tangles: Together at Last?

Whether it is amyloid plaques or tau tangles that primarily cause neurodegeneration is highly controversial. Recently, however, two groups of scientists present evidence that links tau aggregation to amyloid plaques. In one case, transgenic mice expressing mutant tau protein were crossbred with transgenic mice expressing mutant α -amyloid precursor protein. Although these mice developed amyloid plaques at the same age, the levels of neurofibrillary tangles increased significantly²⁷.

Similarly, to link amyloid plaques to tau tangles, another group injected A β fibrils into brains of transgenic mice expressing mutant tau²⁸. Findings revealed that there is a fivefold increase in the number of neurofibrillary tangles, and they begin to appear shortly after injection of A β fibrils²⁸. Thus, there is a connection between amyloid plaques and tau tangles, but how overproduction of A β causes tau dysfunction is unclear. Also, it needs to be assessed whether these mice develop AD symptoms such as dementia. Nevertheless, these models provide a means to develop therapies that can target amyloid plaques and tau tangles^{27, 28}.

Like Tau, Like α -Synuclein

Like tau, α -synuclein is part of the abnormal protein aggregates found in PD. The familial form of PD, with an autosomal-dominant inheritance pattern, was first linked to a missense mutation (Ala³⁰Thr) in α -synuclein by Polymeropoulos et al²⁹. Next, our lab discovered a link between α -synuclein and Lewy bodies, which are characteristic of PD. Substantia nigra neurons from PD patients stained positive for Lewy bodies containing α -synuclein³⁰.

We then moved on to demonstrate that wild type and mutant α -synucleins (Ala³⁰Pro and Ala⁵³Thr) self-aggregate *in vitro*³¹. The solubility of wild type and mutant α -synucleins decreased, and they formed into elongated filaments. α -Synuclein polymerization was concentration and time dependent, suggesting that

initial conformational changes in α -synuclein might serve as seeds for advanced polymerization³¹. Also, the A53T mutant α -synuclein was most prone to aggregate, and it may be a more pathogenic species than wild type or A30P α -synuclein³¹. When immunogold labeling was done, the N- and C-terminal regions of α -synuclein were less accessible and weakly labeled, thus implying that these regions are involved in polymer formation and are buried within filaments³¹.

Synaptic Role of α -Synuclein

Although the functions of α -synuclein are not clearly understood, our lab has attempted to characterize the role of this protein. Using neuronal cultures, we found that expression of α -synuclein was localized primarily in presynaptic vesicles³². Furthermore, antisense inhibition of α -synuclein resulted in decreased pools of synaptic vesicles at the presynaptic terminal. Also, we observed a decrease in levels of synapsin I and synaptophysin, proteins found in synaptic terminals³². Our results suggest that one function of α -synuclein might include maintenance of synaptic vesicle pools.

Modeling PD in Mice and Flies

To prove that α -synuclein aggregates are pathogenic, our lab created transgenic mice that expressed A53T mutant and wild type α -synuclein. Transgenic mice expressing the A53T mutant developed neuropathological symptoms similar to PD patients³³. These included accumulations of α -synuclein inclusions and severe movement disorders. Thus, formation of filamentous α -synuclein inclusions correlates with disease onset³³.

Other labs have used *Drosophila* models to recapitulate features of PD³⁴. Transgenic flies overexpressing wild type and mutant α -synuclein displayed degeneration of dopamine neurons, formation of α -synuclein inclusions, and motor impairments³⁴.

Our lab has also used *Drosophila* to study factors that enhance or suppress PD pathology³⁵. In these models, dopaminergic neuronal loss was prevented by expression of the molecular chaperone Hsp70. Although toxicity of α -synuclein was suppressed in transgenic flies, no change in aggregate pathology was evident³⁵. Also, interference with endogenous chaperone activity enhanced toxicity of α -synuclein³⁵. In addition, molecular chaperones were found to be present in Lewy bodies of PD patients³⁵.

Our data strongly suggests that molecular chaperones might help prevent degeneration of dopaminergic neurons by suppressing α -synuclein. On the other hand, α -synuclein toxicity might be enhanced due to the trapping of chaperones in Lewy bodies³⁵. Increasing the activity of molecular chaperones might prove to be an effective treatment in PD patients³⁵. It has yet to be determined, however, whether expression of Hsp70 reverses PD symptoms, such as motor impairments, in these transgenic flies.

α -Synuclein Caught in a Triangle

Along with mutations in the α -synuclein gene, familial PD has been associated with mutations in two other genes, both of which are gaining considerable importance. Mutations in the parkin gene cause autosomal recessive PD, where the onset of PD is seen at an earlier age³⁶. As ubiquitin is known to be present in α -synuclein Lewy bodies, one model hypothesizes that the parkin protein regulates the ubiquitin degradation pathway of α -synuclein³⁷.

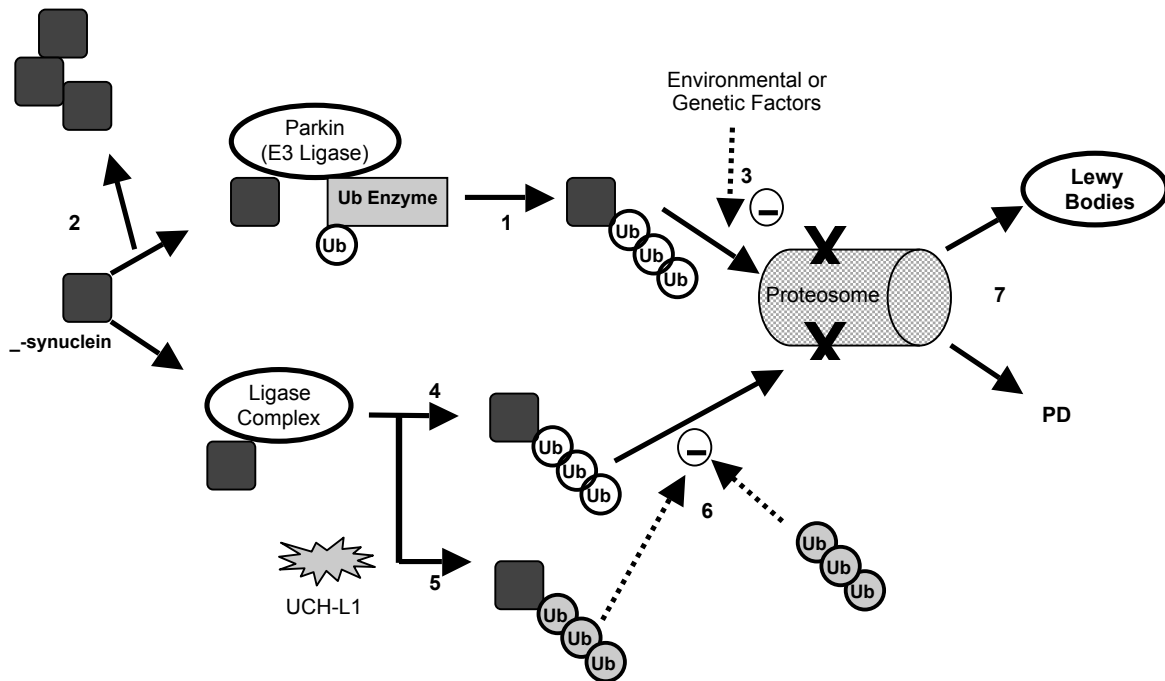


Figure 2. Proposed Models for Parkin and UCH-L1 Interaction with α -Synuclein

The parkin ubiquitin complex polyubiquitinates α -synuclein and prepares it for degradation (1). Other environmental or genetic factors might inhibit proper degradation of α -synuclein, causing accumulation and fibrillization of α -synuclein in Lewy bodies (3). Mutations in parkin can cause accumulation of nonubiquitinated α -synuclein (2). Loss of control of UCH-L1 ligase activity could cause extension of polyubiquitin chains on α -synuclein and inhibit its degradation (5). These ubiquitin additions require no ATP and are thus different from ubiquitin additions by ligase complexes (4) (difference in Ub is indicated by gray or white color). Polyubiquitin chains themselves can inhibit proteasomal degradation (6). Thus, in both cases, accumulation of ubiquitinated α -synuclein can cause formation of Lewy bodies or PD (7).

Recently, parkin was identified as an E3 ubiquitin ligase that associates with a ubiquitin enzyme and a distinct form of α -synuclein³⁷. Whereas normal parkin ubiquitinates α -synuclein, mutant parkin failed to bind α -synuclein and caused an accumulation of nonubiquitinated α -synuclein³⁷. Therefore, it is proposed that loss of parkin function leads to pathological accumulation of α -synuclein, neuronal loss, and an absence of ubiquitin-tagged Lewy bodies, a common feature of autosomal recessive PD patients. Wild-type parkin is thought to polyubiquitinate α -synuclein and prepare it for proteasome degradation; however, interference by other genetic or environmental factors might prevent degradation of these proteins and lead to formation of Lewy bodies³⁷ (Figure 2).

A gene that has been linked to autosomal dominant PD is ubiquitin C-terminal hydrolase-L1 (UCH-L1)³⁸. UCH-L1 was recently shown to possess ligase activity that inhibited proteasomal degradation of α -synuclein³⁹. UCH-L1 is capable of extending the polyubiquitin chain of α -synuclein by ATP-independent addition of ubiquitin molecules. This process differs from ubiquitylation by other ligases, such as E3, that require ATP to be present³⁹. Furthermore, it is hypothesized that polyubiquitinated α -synuclein or polyubiquitin chains themselves inhibit proteasome degradation. Inhibition consequently promotes aggregation and fibril formation of α -synuclein pathogenic for PD³⁹ (Figure 2).

Untangling the Problem

Although research of neurodegenerative diseases has made great advances over the past decade, the mechanisms of neurodegeneration are far from being untangled. Studying the role of tau and α -synuclein in pathogenesis of FTDP-17, AD, and PD will allow

development of effective therapies and treatments. Although it is unclear how tau and α -synuclein abnormalities lead to neurodegeneration, many studies have provided evidence for probable pathways.

Investigating tau mutations in FTDP-17 has established that hyperphosphorylated tau aggregates are sufficient to cause neurodegeneration. Events leading to hyperphosphorylation of tau in AD can include an imbalance of phosphatase or kinase activity. Emerging evidence links tau tangles to amyloid plaques; however, how amyloid plaques lead to increased tau dysfunction has yet to be established.

In PD, filamentous α -synuclein in Lewy bodies have been linked to the onset of neurodegenerative phenotypes. It is still unclear how pathogenic α -synuclein aggregates cause dopaminergic cell death. Models have also been proposed for how mutant α -synuclein, parkin and UCH-L1 genes cause PD.

Transgenic models made by our lab and others reproduce important features of FTDP-17, AD, and PD, and offer insights into pathogenesis of these diseases. Development of additional animal models will be beneficial in finding potential cures and treatments for neurodegenerative disorders.

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