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Forum Review

Role of Oxidative Damage in Protein Aggregation Associated with Parkinson's Disease and Related Disorders

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ABSTRACT

Parkinson's disease, the most common movement disorder, is characterized by the loss of brainstem neurons, specifically dopaminergic neurons in the substantia nigra, as well as the accumulation of neuronal cytoplasmic filamentous proteinaceous inclusions comprised of polymerized α-synuclein. It was reported recently that α-synuclein can induce the formation of filamentous tau inclusions, which are characteristic of disorders like Alzheimer's disease and Lewy body variant of Alzheimer's disease, suggesting that a similar mechanism may exist between α-synuclein fibrillogenesis and tau polymerization. Pathological brain inclusions comprised of α-synuclein or tau proteins are associated with a spectrum of neurodegenerative disorders, and oxidative and nitrative injury has been implicated in all of these diseases. However, the role of oxidative damage in α-synuclein and tau polymerization and pathological inclusion formation is complex. Differences in the level, type, and temporal sequence of the oxidative alterations appear to result in both inhibitory and stimulatory effects on the fibrillogenesis of these proteins. *Antioxid. Redox Signal.* 7, 673–684.

PARKINSON'S DISEASE

Parkinson's disease (PD), first described as the "shaking palsy" by James Parkinson in 1817, is the most common movement disorder and the second most common neurodegenerative disorder. It affects over one million people in North America alone (74) and influences all races and both sexes. The prevalence of PD increases with age; 0.5–1% of the population from 65 to 69 years of age is diagnosed with PD, and this percentage increases to 4% at the age of 85 years (120). Some studies have indicated that there may be a greater prevalence for PD in populations from rural, farming communities than from city-dwelling or suburban areas, suggesting that undefined environmental exposures may be a risk factor (5, 80, 107).

PD is clinically defined by four characteristic motor symptoms: uncontrollable resting tremor, slowness in movement (bradykinesia), postural instability, and muscle rigidity (60, 109). The primary motor discoordination in PD has been largely attributed to a progressive and extensive loss of dopaminergic neurons in the substantia nigra pars compacta, which results in greatly decreased dopamine levels in the striatum (19, 74, 96). Pharmacological replacement of this neurotransmitter with its precursor L-dopa is widely used to treat some of the symptoms of PD, and responsiveness to L-dopa is another criterion used in clinical diagnosis (109). However, dopaminergic neurons are not uniquely affected in PD as neuronal loss can occur in other brain nuclei, including the locus ceruleus, basal nucleus of Meynert, and raphe nuclei, which likely contributes to other associated neurological clinical presentations (85, 129).

In addition to the clinical features of PD and the extensive loss of dopaminergic neurons in the substantia nigra, other diagnostic neuropathological features characterize this disease. PD brains display the presence of intracytoplasmic inclusions known as Lewy bodies (LBs) in some of the remaining dopaminergic neurons, variable extracellular melanin released from degenerating neurons, and gliosis (17, 33, 34). LBs were first described in cholinergic neurons of the substantia innominata by Friederich Lewy in 1912, but their

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presence in dopaminergic neurons of the substantia nigra pars compacta was first noted by Trétiakoff in 1919. These pathological lesions now known as "classical" LBs are eosinophilic and usually have a distinctive laminated spheroidal appearance (see Fig. 1A). Ultrastructurally, they are comprised of a halo of radiating fibrils (7–25 nm in diameter), often referred to as the "corona," which surrounds a matted meshwork of filaments intertwined with amorphous material at the "core" (30, 34, 59, 104). LBs are not restricted to the substantia nigra in PD as they also exist in many other brainstem nuclei and diencephalic regions (34).

α-SYNUCLEIN AND LEWY BODIES

From genetic studies, α-*synuclein* was the first gene to be directly linked to PD (101). In the seminal report by Polymeropoulos and colleagues, the Ala53Thr mutation resulting from a G to A transition at position 209 of the α-*synuclein* gene was identified in a large Italian family (the Contursi kindred) and three small possibly related Greek families with autosomal dominant PD (101). Shortly following the report of the Ala53Thr mutation in α-synuclein, a series of studies convincingly demonstrated that α-synuclein is the building block of the ~10-nm filamentous polymers that form LBs (for reviews, see 27, 47). Moreover, the Ala53Thr mutation in α-synuclein has now been identified in at least eight additional families (4, 82, 97, 117). Two other mutations in α-synuclein, Ala30Pro and Glu46Lys, have also been identified in kindreds with PD (73, 135). A short trisomy of chromosome 4 that includes the α-*synuclein* gene has also been identified as the cause of disease in some families with dementia with LBs (DLB), likely due to increased expression of α-synuclein protein (32, 110).

Although LBs are often emphasized in the description of PD for historical and histological reasons, α-synuclein inclusions in neuronal processes are much more abundant than LBs (28, 90), and they are likely to play a much greater role in perturbing neuronal function. These aberrant inclusions in neuronal processes were first described as dystrophic ubiquitin-positive neurites termed Lewy neurites (LNs) (9, 22), but larger inclusions that cause a distension of axons are also known as neuroaxonal spheroids.

SYNUCLEINOPATHIES: A SPECTRUM OF DISEASES WITH α-SYNUCLEIN PATHOLOGICAL INCLUSIONS

Shortly following the first report of a mutation in the α-*synuclein* gene, a series of studies demonstrated and confirmed that filamentous α-synuclein was the major component of several types of pathological inclusions characteristic of many disorders (65, 114–116, 119, 121). The term synucleinopathies was coined to characterize this group of diseases, which often contain a much larger distribution of α-synuclein inclusions throughout the neuroaxis compared with PD. Although the detailed descriptions of these diseases are beyond the scope of this review, some major features that highlight the importance of intracytoplasmic pathological lesions will be discussed.

Diseases with widespread LBs and LNs

In some LB diseases, inclusions are not predominantly concentrated in brainstem nuclei, but are distributed throughout the neuroaxis as well. In most CNS regions other than the brainstem, LBs present as smaller lesions similar to the cortical LBs first described by Okazaki *et al.* in 1961 (93). These

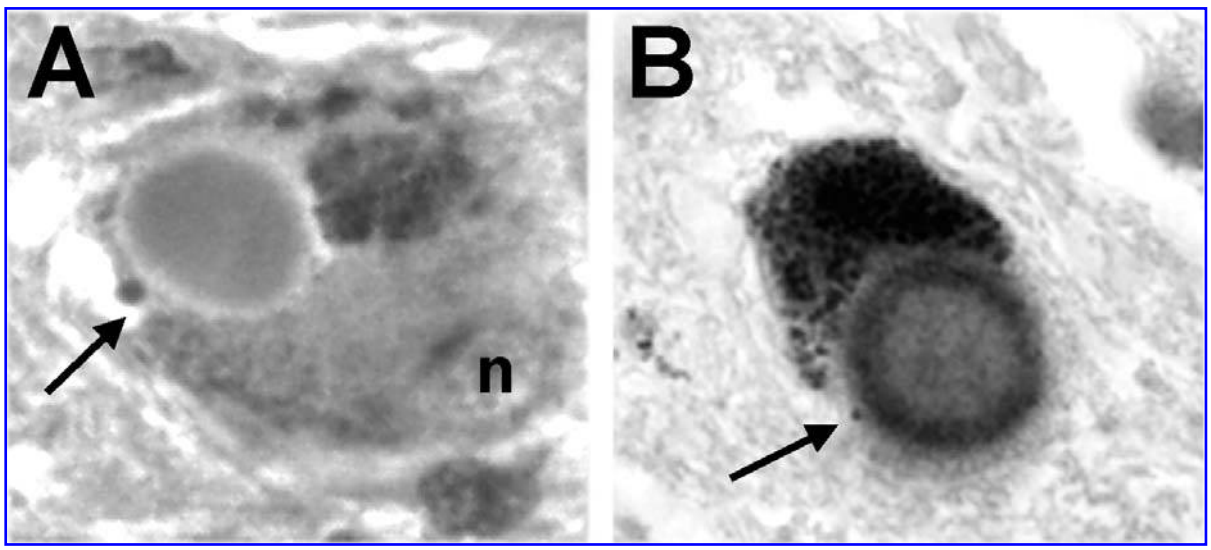

FIG. 1. Lewy bodies (LBs) in the substantia nigra are modified by 3-nitrotyrosine. (A) A classical LB stained with hematoxylin and eosin in a dopaminergic neuron in the substantia nigra pars compacta of a patient with PD. **(B)** A classical LB labeled with an antibody specific for nitrated α-synuclein in a dopaminergic neuron in the substantia nigra pars compacta of a patient with DLB. "n" identifies the nucleus. Arrows point to LBs.

inclusions have less clearly defined morphology and have inconspicuous appearance by hematoxylin and eosin staining.

Most patients with cortical LBs are typically affected by parkinsonism, dementia, and many other complex clinical features (86). This condition has been termed dementia with LBs (DLB), although many other appellations have also been used. Patients with DLB usually display dementia with subsequent parkinsonism, although a significant percentage (~25–40%) of cases initially display parkinsonism followed by dementia (71, 79). DLB can present in a "pure" form where LBs are the major pathological lesions. However, these cases are typically less common because the majority of DLB brains typically have sufficient Alzheimer's disease (AD) pathology (*i.e.*, neurofibrillary tangles and senile β-amyloid plaques) for a concurrent diagnosis of AD and DLB (54, 71), which is typically termed LB variant of AD (LBVAD). Importantly, the involvement of cortical LBs in the pathogenesis of cognitive decline is suggested by the correlation between the severity of dementia and the density of LBs in patients with DLB (55, 62, 79, 84, 103). Furthermore, the development of anti-α-synuclein antibodies that are selective for pathological forms of α-synuclein or the use of proteinase K retrieval has revealed an unanticipated high abundance of LNs (28, 90), indicating that the presence of LNs is usually underappreciated and that this type of pathology is likely to contribute significantly to neuronal dysfunction.

Fibrillar α-synuclein inclusions are important findings in several other diseases. For example, they can be abundant in patients with neurodegeneration with brain iron accumulation type 1 (NBIA-1; previously known as Hallervorden–Spatz disease). This rare progressive neurodegenerative disorder occurs in both a familial and sporadic form, and it is clinically characterized by parkinsonism features, pyramidal signs, seizures, and mental deterioration that can culminate in dementia (23). The histopathological findings defining NBIA-1 are neuroaxonal spheroids (*i.e.*, 20–100-μm-wide axonal swelling), which appear to consist mainly of α-synuclein (2, 36, 91, 121), and intense iron deposits in the globus pallidus and substantia nigra pars compacta. The recessive familial form of this disease has been attributed to the defects in the *pantothenate kinase 2* gene (136). Although LBs have been reported in the majority of the brains of NBIA-1 patients, some brains do not appear to display these lesions (see 102 and references therein).

Multiple system atrophy: a neuronal and oligodendritic synucleinopathy

Multiple system atrophy (MSA) is an adult-onset neurodegenerative disease characterized by varying degrees of parkinsonism, cerebellar ataxia, and autonomic dysfunction (46, 127, 128). MSA is characterized histologically by the accumulation of filamentous proteinaceous inclusions in the form of neuronal cytoplasmic inclusions (NCIs) and glial cytoplasmic inclusions (GCIs) in oligodendrocytes (70, 98). MSA brains show varying degrees of atrophy and demyelination of the cerebellum, pons, and medulla, as well as the loss of pigmented neurons of the substantia nigra. GCIs usually appear as flame- or sickle-like inclusions in oligodendrocytes that can be readily detected by Gallyus silver staining (98). GCIs can be found throughout the white matter, but the greatest abundance of these inclusions occurs in the basal ganglia, the substantia nigra, the pontine nucleus, medulla, and cerebellum (3, 26, 75). Ultrastructurally, GCIs and NCIs are predominantly composed of a meshwork of randomly arranged, loosely packed filaments of polymerized α-synuclein (26, 115, 121, 125).

α-SYNUCLEIN BIOLOGY

Three neuronal synuclein proteins (termed α-, β-, and γ-synuclein) have been identified in humans (11, 38), but herein only α-synculein will be discussed, because it is the only synuclein protein directly implicated in neurodegenerative diseases. α-Synuclein is a relatively small protein of 140 amino acids. Its most striking features consist of six imperfect repeats (KTKEGV) distributed throughout most of the N-terminal half of the protein and an acidic C-terminal region (68) (see Fig. 2A). α-Synuclein is a heat-stable protein that is "natively unfolded," such that it has no apparent defined structure (20, 126), and it is predominantly expressed in the neurons of the CNS, where it is localized at presynaptic terminals (39, 67, 68, 131). The function of α-synuclein still remains unknown, but several clues suggest that it is involved in modulating synaptic transmission and neuronal plasticity. Electron microscopy studies have localized α-synuclein in close proximity to synaptic vesicles at axonal termini (39, 67), and biochemical analysis has revealed that a small fraction of α-synuclein may be associated with vesicular membranes, although the majority of this protein is cytoplasmic (39, 64). It is predicted that α-synuclein can form amphipathic helices that can associate with vesicular membranes (39), and indeed, an increase in α-helical structure is observed upon binding to small synthetic unilamellar vesicles *in vitro* (20). It has been suggested that α-synuclein may be involved in neuronal plasticity (39), but it does not play a role in initial synaptic formation (87, 131). Ablation of α-synuclein by engineering a null mutation in mice is not associated with any overt phenotype, but results in a subtle alteration in the recovery period following induced repetitive synaptic vesicular release (1). Furthermore, the reduction of α-synuclein levels in hippocampal neurons results in a significant reduction of the distal pool of synaptic vesicle (12, 87). Hence, these results demonstrate that α-synuclein can modulate vesicular synaptic function, but the precise mechanism is still unclear.

MECHANISMS OF α-SYNUCLEIN POLYMERIZATION AND INCLUSION FORMATION

Recombinant human α-synuclein can readily assemble into elongated homopolymers *in vitro.* The widths of these fibrils are consistent with those sarcosyl-insoluble fibrils isolated from human diseased brains and filaments visualized directly in LBs and GCIs (13, 31, 40, 89). Polymerization is associated with a concomitant change in secondary structure from random coil to anti-parallel β-pleated sheet structure consistent with the Thioflavine-S reactivity of these filaments

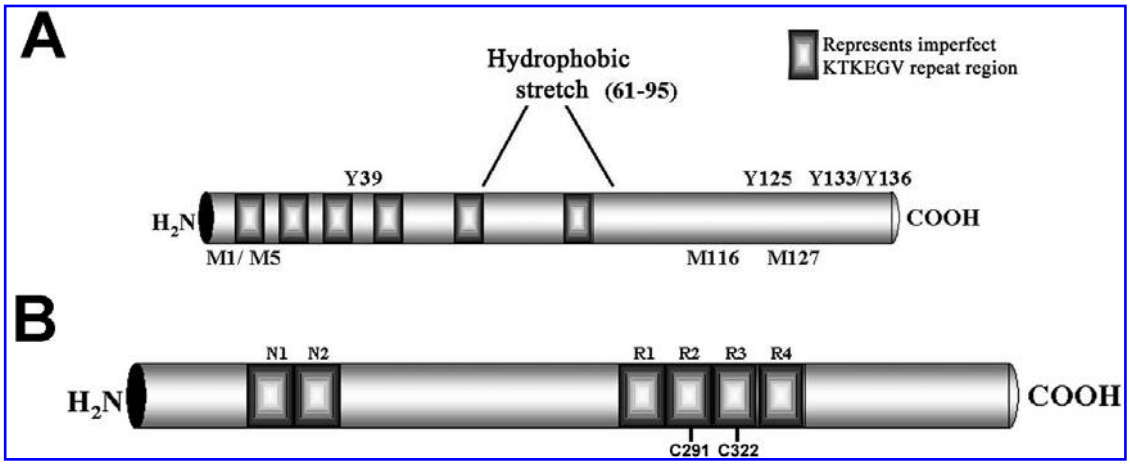

FIG. 2. Diagram of α-synuclein and tau proteins. (A) Diagram of α-synuclein protein. This protein has six degenerate KTKEGV repeats throughout the N-terminal region and a hydrophobic stretch that spans the middle region. Note the four methionine (M) and four tyrosine (Y) residues. **(B)** Schematic of tau protein. The longest isoform of tau has two N-terminal inserts (N1 and N2) and four microtubule-binding regions (R1–R4). Due to alternative splicing of the two N-terminal inserts and R2, six isoforms are normally expressed in the CNS. Isoforms with four repeats have two cysteine (C) residues, but isoforms without R2 have only one cysteine residue.

(14, 42, 89, 108). One of the rate-limiting steps in the polymerization of α-synuclein appears to be the formation of "seeds" or protofibrils (15, 132), and although controversial, it has also been suggested that some types of protofibrils may have toxic properties (124) (see below).

Several molecular changes have been shown to influence the rate of α-synuclein fibrillogenesis. Directly associated with the pathogenic mechanism, the Ala53Thr α-synuclein mutation accelerates the rate of fibrillogenesis (13, 31, 40, 89). This mutation also affects the ultrastructure of the polymers in that the filaments are slightly wider and more twisted in appearance (13, 31, 40). The Ala30Pro mutation may also modestly increase the propensity of α-synuclein to polymerize (31, 89), but this finding has not been reported consistently (40, 108). These discrepancies may be due to technical differences in methods for assessing α-synuclein polymerization, because the Ala30Pro mutation may increase the propensity to form small oligomers rather than filaments (15, 108). Ablation of the C-terminal region of α-synuclein increases the propensity of the protein to form fibrils (18, 88, 108). Although the pathological implications of the latter finding are still unclear, it is possible that the aberrant proteolysis of α-synuclein may promote the formation of "seeds" that could initiate α-synuclein filament assembly. Further, structure–function analysis of α-synuclein demonstrated that the hydrophobic middle region of the protein is required for filament formation (42). Moreover, β-synuclein lacks the ability to form fibrils, because it is deficient in an 11-amino acid stretch within this hydrophobic region. In contrast, γ-synuclein, which has a hydrophobic region similar to α-synuclein, is also largely deficient in the ability to polymerize due to specific amino acid differences in the hydrophobic middle section (24). Phosphorylation of Ser129 in α-synuclein, which is a substrate for casein kinase II, also can promote the assembly of α-synuclein into fibrils *in vitro* (35). Oxidative-induced alterations can also influence α-synuclein polymerization, but the outcomes are more complex (see below).

A ROLE FOR α-SYNUCLEIN IN TAU FIBRILLOGENESIS AND INCLUSION FORMATION

Tau is predominantly a neuronal microtubule-binding protein that stabilizes and promotes microtubule polymerization in neuronal perikarya and processes (10, 77). In the adult human brain, six isoforms ranging between 352 and 441 amino acids in length are produced as a result of alternative mRNA splicing from a single gene on chromosome 17 (10, 77) (see Fig. 2B). Although monomeric tau proteins are soluble molecules, they can polymerize to form fibrils known as paired helical filaments and straight filaments that are the building blocks of diverse types of intracytoplasmic pathological inclusions such as neurofibrillary tangles in AD and LBVAD (10, 77). Similar to those in α-synuclein, mutations in the *tau* gene are associated with neurodegenerative disease, in this case known as frontotemporal degeneration with parkinsonsim linked to chromosome-17 (FTDP-17), and tau pathological inclusions are present in a spectrum of diseases collectively termed tauopathies (10, 63, 77). However, unlike α-synuclein, tau requires cofactors (*e.g.*, polyanions such as glycosaminoglycans or nucleic acids) to fibrillize (49, 69). Recently, α-synuclein has also been shown to facilitate tau polymerization *in vitro* and the formation of tau inclusions in mice and humans (45). The ability of α-synuclein to induce tau pathology is consistent with the frequent presence of both tau and α-synuclein inclusions in many brains with neurodegenerative diseases and sometimes within the same lesions

where tau and α-synuclein fibrils occasionally appear to be intertwined (45, 66, 78, 81, 83, 105).

TOXICITY OF FILAMENTOUS α-SYNUCLEIN INCLUSIONS?

Although the formation of α-synuclein inclusions is clearly associated with the etiology of many neurodegenerative diseases, the nature of the toxic polymerized species of α-synuclein is still debated. Evidence from *in vitro* studies, transgenic *Drosophila* and mouse models, and the analysis of autopsy specimens indicates that the aberrant polymerization of α-synuclein into filaments, which eventually form large intracytoplasmic inclusions, can lead to the dysfunction and demise of neurons or oligodendrocytes (21, 47). Some of this evidence includes the increased propensity of the Ala53Thr mutant of α-synuclein to fibrillize *in vitro*, as well as the widespread and abundant distribution of α-synuclein pathology in individuals carrying the Ala53Thr α-synuclein mutation associated with neurodegeneration (13, 29, 40, 89). Similarly, expression of Ala53Thr human α-synuclein in mice can result in the formation of abundant α-synuclein inclusions that is accompanied by severe neuronal dysfunction and axonal degeneration that leads to death (21, 43, 76). Also indicative of the toxic role of α-synuclein inclusions is the association between the presence of GCIs and MSA. These abundant α-synuclein inclusions are associated almost exclusively with MSA, and they can nearly fill the entire cytoplasm of oligodendroctyes (115, 121, 125). Therefore, it is likely that α-synuclein inclusions can impair cellular function by obstructing normal cellular trafficking, disrupting cell morphology, and trapping other cellular components, which all eventually can lead to cell death.

Although the preponderance of evidence supports the notion that α-synuclein toxicity is linked to the formation of filamentous pathological inclusions, Lansbury and colleagues have supported the alternative, but not mutually exclusive, concept that protofibrils may be toxic (50). This possibility stems from an analogy to the toxic protofibril hypothesis of the amyloid-β peptide involved in AD (50). The evidence for the toxic nature of α-synuclein protofibrils relies predominantly on *in vitro* data (50). Further, mutant Ala30Pro α-synuclein was reported to have a tendency to accumulate as protofibrils instead of mature fibrils (15, 50). However, the toxic nature of Ala30Pro α-synuclein protofibrils has not been validated *in vivo* (21, 90). Nevertheless, it has been proposed that the formation of α-synuclein protofibrils with an annular appearance may integrate into membranes, resulting in the formation of pores that could cause uncontrolled membrane permeability (50, 124).

LINKING OXIDATIVE/NITRATIVE DAMAGE TO SYNUCLEINOPATHIES AND THE FORMATION OF PATHOLOGICAL LESIONS

The notion that oxidative injury plays an important role in PD, as well as other neurodegenerative diseases, has been supported by numerous findings (for reviews, see 7, 44). In particular, relevant to the selective vulnerability of dopaminergic neurons is the permanent heightened levels of free radicals in dopaminergic neurons due to dopamine metabolism and autoxidation (52, 53, 58, 112). The normal enzymatic metabolism of dopamine results in the generation of hydrogen peroxide (H_2O_2), and the nonenzymatic autoxidation of dopamine results in the formation of reactive quinones and semiquinones that generate H_2O_2, superoxide anions, and hydroxyl radicals (53, 58). It also has been suggested that dopamine oxidation that forms 6-hydroxydopamine can readily undergo rapid autoxidation with molecular oxygen to generate reactive free radical species (53). These properties of dopamine are thought to render dopaminergic neurons more vulnerable to oxidative insults, but there is also ample evidence of oxidative stress in other disease-specific affected areas associated with neurodegenerative diseases with α-synuclein and tau pathological inclusions (for review, see 44). However, the issues of whether and how oxidative alterations affect the polymerization of α-synuclein and tau are complex and appear to be dependent on the extent and type of modification, and the temporal sequence at which oxidative damage occurs.

α-Synuclein in pathological lesions is modified extensively by 3-nitrotyrosine (25, 41, 51) (see Fig. 1B). The finding that nitrated α-synuclein is detected exclusively in the detergent-insoluble fraction from diseased human brain samples (41) supports the involvement of nitrative damage in the formation of pathological inclusions. However, *in vitro* experiments showed that nitration of recombinant α-synuclein protein with either peroxynitrite or tetranitromethane inhibits filament formation (92, 134). Moreover, although nitration of Tyr residues reduces the propensity of α-synuclein to fibrillize, it cannot be the only modification involved because the polymerization of Tyr-less α-synuclein (all four Tyr residues have been mutated to Phe) is inhibited by peroxynitrite, albeit to a much lesser extent (92). However, the exposure of cultured cells to nitrative conditions can result in the formation of filamentous intracytoplasmic α-synuclein inclusions by a mechanism that has not been clearly delineated (92, 99). Nevertheless, *o,o'*-dityrosine, the other major modification resulting from exposure to peroxynitrite, has been implicated in the formation of stable inclusions, as the formation of *o,o'*-dityrosine on preformed fibrils can greatly stabilize these polymers (113). The presence of *o,o'*-dityrosine in α-synuclein can also assist as a nucleation unit in facilitating polymerization (72).

α-Synuclein does not have any Cys or Trp residues, which typically are modified easily by oxidation. However, α-synuclein has four Met residues. Exposure of α-synuclein to a high concentration (1.2 *M*) of H_2O_2 oxidizes all four Met residues and prevents fibrillization of the protein (123). However, lower levels of H_2O_2 (300 μM), even in the presence of transition metals, does not prevent filament formation (92). The exposure of α-synuclein to high concentrations of H_2O_2 does not permanently render α-synuclein polymerization incompetent, as the subsequent addition of certain metals, such as Ti^{3+}, Zn^{2+}, Al^{3+}, and Pb^{2+}, appears to affect the folding of α-synuclein and reestablish the ability of α-synuclein to fibrillize (133). Therefore, the exposure to strong oxidative conditions may lead to the folding of α-synuclein in a conforma-

tion that is not conducive to polymerization (see Fig. 3A). It remains to be determined whether exposure to peroxynitrite or tetranitromethane can also partially inhibit filament formation through a similar mechanism.

The presence of dopamine also can inhibit α-synuclein filament formation *in vitro* by stabilizing α-synuclein in a protofibrillar state (16), although the mechanism has not been clearly elucidated. This process requires oxidation and is associated with the formation of dopamine adducts (16). However, the requirement for these adducts is unclear, and it is possible that oxidation induced by dopamine inhibits filament formation through a mechanism similar to that described above (see Fig. 3A). The addition of dopamine-modified α-synuclein to unmodified α-synuclein also prevents filament formation, suggesting that a small amount of modified or "misfolded" α-synuclein can have an important inhibitory effect on the kinetics of α-synuclein polymerization (16).

It has been demonstrated that exposure of α-synuclein to oxidative or nitrative conditions results in sodium dodecyl sulfate-stable cross-linked oligomers (92, 94, 95, 122). However, if these modifications occur prior to fibrillogenesis, they can result in the formation of α-synuclein aggregates (56, 57) that may not be permissive for fibril formation. Conversely, oxidative cross-linking of preformed α-synuclein fibrils can stabilize the protein in a fibrillar state (92). Interestingly, the type of cross-links resulting from transition metal/H_2O_2 oxidation has not been resolved. α-Synuclein has no Trp or Cys residues, and α-synuclein protein where all four Tyr residues have been mutated to Phe is still susceptible to cross-linking by these agents. Cross-linking might occur through the direct interaction of carbon radicals derived from α-hydrogen abstraction by hydroxyl radicals or by Schiff base condensation with carbonyls converted from basic amino acids (118). A better understanding of this process is important, because it is likely that stabilizing modifications can promote the formation of pathological inclusions, and cross-linking may render these inclusions resistant to cellular degradation mechanisms.

Similar to α-synuclein, there is evidence that oxidative and nitrative damage can affect the ability of tau to form pathological inclusions (61, 111). Tau molecules have one or two Cys residues, depending on the tau isoforms (48) (see Fig. 2B). Tau molecules with three microtubule-binding repeats have only one Cys residue at position 322 (numbered accord-

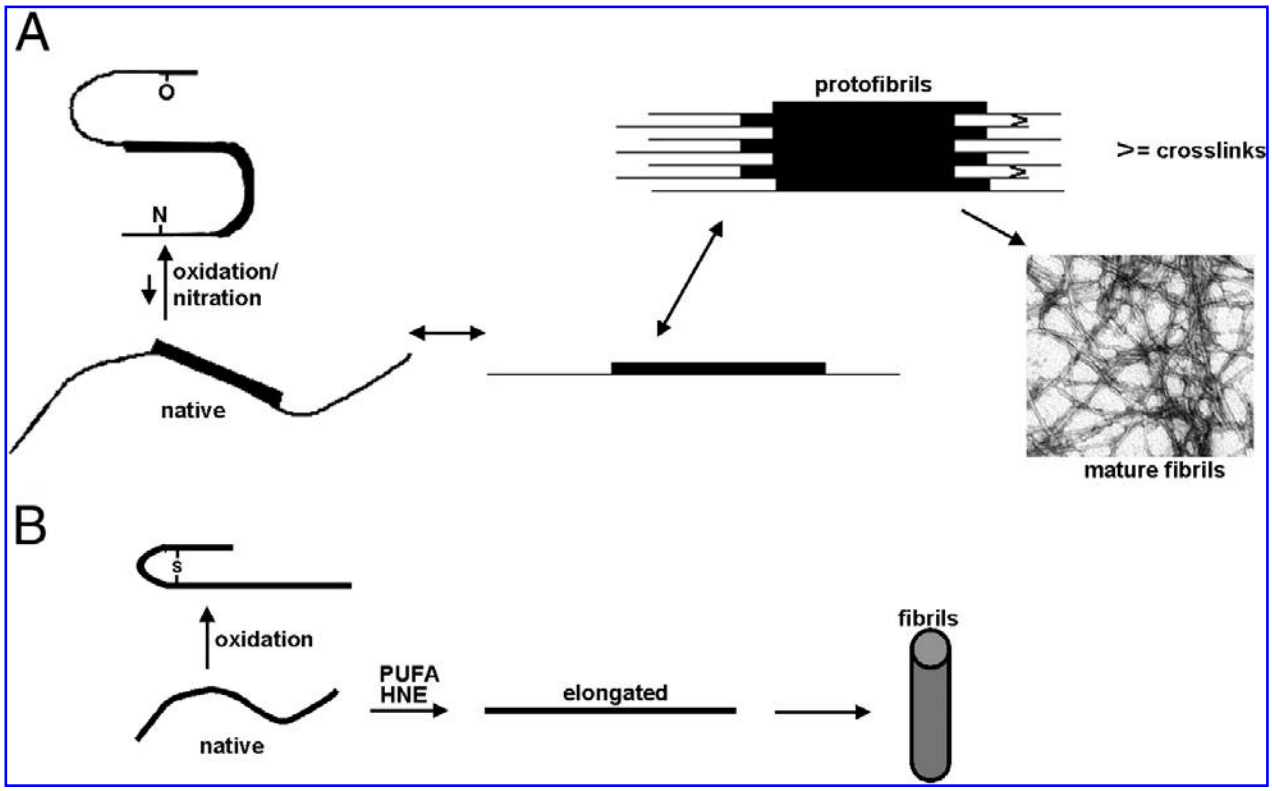

FIG. 3. Schematic of oxidative pathways that can influence α-synuclein and tau polymerization. (A) Model of the effects of oxidation and nitration on α-synuclein polymerization. Native α-synuclein exists predominantly in an unstructured conformation. Exposure to strong oxidants or dopamine may promote a conformational change that is not permissive for filament formation. This conformation is reversible, and under some conditions, the protein can adopt a conformation that allows polymerization. At later stages of polymerization, nitrative or oxidative cross-linking can stabilize the polymers, promoting the formation of mature fibrils. **(B)** Schematic of the effects of some oxidative process on tau polymerization. Free tau exists in an unstructured conformation. Oxidation of 4R-tau can result in intramolecular disulfide bonds that prevent the protein from polymerizing. The addition of several types of polymerization inducers, including 4-hydroxy-2-nonenal (HNE) or polyunsaturated fatty acids (PUFA), can change the conformation of tau, promoting its elongation into fibrils.

ing to the longest tau isoform). Tau molecules with four microtubule-binding repeats have two Cys residues at positions 291 and 322. The formation of intermolecular disulfide bridges at either Cys291 or Cys322 can accelerate tau fibrillogenesis induced by heparin, likely by promoting the required alignment of molecules for polymerization (6, 8, 106). However, intramolecular disulfide cross-links, which can only occur for isoforms with four repeats, prevent filament formation by stabilizing the protein in a conformation that is not permissible for fibril formation (6, 8) (see Fig. 3B).

Further evidence that oxidative changes can mediate tau fibrillogenesis is the observation that polyunsaturated free fatty acids, which are sensitive to oxidative changes and promote oxidation in other molecules, also can greatly stimulate tau polymerization *in vitro* (130). Further, free radical scavengers prevent tau polymerization induced by polyunsaturated fatty acids, demonstrating that oxidative changes are necessary for this process (37). However, the details of how fatty acid oxidation promotes tau polymerization are not known. Also, 4-hydroxy-2-nonenal, one of the major products of lipid peroxidation, has also been shown to promote the fibrillogenesis of phosphorylated tau (100).

Nitrative injury may also be involved in the formation of tau pathological inclusions. Tau pathological inclusions is modified by 3-nitrotyrosine in a temporal sequence, suggesting this modification occurs early in inclusion formation (61). Exposure of cultured cells to nitrative agents can induce the formation of filamentous inclusions (61), but the mechanism has not been explored. The effects of nitration on tau filament formation *in vitro* and microtubule binding have not been determined.

FUTURE DIRECTIONS

Several important issues concerning the effects of oxidative and nitrative damage on α-synuclein and tau fibrillogenesis remain largely unresolved. It is clear that oxidative changes can either promote or inhibit the polymerization of these proteins, depending on the extent of modification and the stage of polymerization. However, some studies have used harsh conditions that may not reflect physiological states. Further characterization of modifications and types of intermediate polymerization species are needed. For example, it is not clear if oxidation mediated by dopamine or dopamine adducts is involved in preventing α-synuclein polymerization. As the polymerization of α-synuclein (into either protofibrils or mature fibrils) and tau clearly is involved in neurodegeneration, better animals models of these processes are needed to obtain a more comprehensive notion of the importance of the various types of modifications for inclusion formation and neurodegeneration.

ABBREVIATIONS

AD, Alzheimer's disease; DLB, dementia with Lewy bodies; GCI, glial cytoplasmic inclusion; H_2O_2, hydrogen peroxide; LB, Lewy body; LBVAD, Lewy body variant of Alzheimer's disease; LN, Lewy neurite; MSA, multiple system atrophy; NBIA-1, neurodegeneration with brain iron accumulation-type 1; NCI, neuronal cytoplasmic inclusion; PD, Parkinson's disease.

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