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Forum Review

α-Synuclein Aggregation: A Link Between Mitochondrial Defects and Parkinson's Disease?

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ABSTRACT

Protein aggregation is a shared feature of many human neurodegenerative diseases and appears to be an inevitable consequence of excessive accumulation of misfolded proteins. Recent studies suggest that accumulation of fibrillar α-synuclein aggregates is associated with Parkinson's disease and other Lewy body diseases. Furthermore, the missense mutations in α-synuclein that are responsible for some early-onset familial types of the disease promote the aggregation process of this protein. Therefore, the mechanism underlying the cellular α-synuclein aggregation is of great importance in understanding the pathogenic process of these diseases. This review summarizes recent advances in our understanding of the mechanisms underlying α-synuclein aggregation and how the mitochondrial dysfunction plays a role in this process. Protein misfolding and aggregation *in vivo* can be suppressed and promoted by several factors, such as molecular chaperones, protein degradation systems, and free radicals. Many of these factors are under the control of normal mitochondrial function, prompting the speculation that mitochondrial dysfunction might cause the accumulation of protein aggregates. Recent studies indeed show that mitochondrial defects can lead to the aggregation of α-synuclein. In addition, potentially toxic effects of α-synuclein have been linked to the aggregated forms rather than the monomers, both *in vitro* and in cultured cells. Therefore, it is postulated that aggregation of α-synuclein might be one of many possible links that connect mitochondrial dysfunction to neurodegeneration. *Antioxid. Redox Signal.* 5, 337–348.

INTRODUCTION

PROGRESSIVE IMPAIRMENT OF MITOCHONDRIAL FUNCTION and increased oxidative stress have been implicated in the pathogenesis of Parkinson's disease (PD) and other neurodegenerative diseases (7, 103). In idiopathic PD, complex I activity of the mitochondrial respiratory chain decreases by 30–40% in the substantia nigra (53, 81, 108). Furthermore, a selective reduction in the immunoreactivity for complex I subunits was reported in the substantia nigra of PD patients, whereas immunoreactivity for other electron-transport complexes was unaltered (46). Cybrid studies provided additional evidence for mitochondrial defects in PD. Cybrids that carried mitochondria derived from platelets of PD patients displayed reduced complex I activity (42, 121). In addition, several groups have reported complex I inhibition and reduction of mitochondrial ATP production by a potent and selective parkinsonian toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, further providing support for the notion that mitochondrial defects are involved in the pathogenesis of PD (92, 109, 117).

PD is pathologically defined by two hallmark features, dopaminergic neuronal loss and Lewy pathologies such as Lewy bodies (LBs) and Lewy neurites (30). Although several mechanisms have been proposed to explain the role of mitochondrial impairment in cell death (for reviews, see 10, 20, 68), the relationship between the Lewy pathologies and mitochondrial defects had not been studied until recently, when fibrillar aggregates of α-synuclein were discovered as major

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components of LBs and Lewy neurites. This review will focus on our current knowledge of the mechanisms of α-synuclein aggregation and discuss the implications of recent findings in the role of mitochondrial impairment in this pathological protein deposition.

α-SYNUCLEIN AND PD

α-Synuclein is 140-amino acid protein that is enriched in neurons in the central nervous system. It is a member of the synuclein protein family, which includes two other members: β- and γ-synuclein (15). All three synucleins have highly conserved N-terminal domains that are composed of imperfect repeats of the consensus sequence KTKEGV. This region is important in the interaction with phospholipid membranes, and upon binding to the membranes, it undergoes a structural transition from random coil to α-helix (22, 25, 99). C-termini of synucleins are enriched with negatively charged amino acids and thought to be important in maintaining solubility of these proteins (123). The mid-region of α-synuclein contains a 12-amino acid motif that is absent in β-synuclein, and this sequence seems to be important for aggregation of this protein (38).

A body of evidence suggests that α-synuclein is normally involved in synaptic transmission. Immunohistochemical studies have shown that α-synuclein is primarily localized in presynaptic terminals (51, 61). In addition, the homozygous deletion of α-synuclein results in a decrease in the size of the "reserve" pools of presynaptic vesicles and impairments in electrophysiological response to a prolonged train of low-frequency repetitive stimulation (13). Finally, another set of α-synuclein knockout mice showed subtle, but clear, abnormalities in nigrostriatal synaptic transmission (1). At a molecular level, it has been suggested that α-synuclein might be involved in lipid trafficking and metabolism (16, 111), regulation of phospholipase D (2, 54), and "chaperoning" protein folding (64, 96, 119). Whether these activities are physiologically relevant and how they are related to the synaptic function remains unknown.

PD is the second most common neurodegenerative disease only to Alzheimer's disease. Clinical symptoms of PD include bradykinesia, rigidity, and resting tremor. Pathologically, the disease is characterized by a loss of nigrostriatal dopaminergic neurons and the presence of LBs (31). The first connection between α-synuclein and PD was made when a missense mutation that was linked to an early-onset type of the disease was identified in an Italian kindred and three Greek families (101). This mutation causes amino acid substitution from alanine to threonine at position 53 (A53T). A more extensive search of mutations in the α-synuclein gene led to the identification of another missense mutation, A30P, in a German family (69). Furthermore, it was quickly found that fibrillar wild-type α-synuclein is a major component of LBs in virtually all LB diseases, including idiopathic PD (6, 120). These findings established that α-synuclein is involved in at least some aspect of PD pathogenesis.

The role of α-synuclein in the pathogenic process has been studied using transgenic animal approaches. Expression of human α-synuclein (wild-type or one of the two mutants, A30P and A53T) in the neurons of transgenic flies produced an age-dependent PD phenotype including fibrillar α-synuclein-containing cytoplasmic inclusions, selective loss of dopaminergic neurons, and a motor deficit (29). In addition, neuronal expression of human α-synuclein in mice generated four transgenic models with neurodegenerative phenotypes. The first transgenic model, developed by Masliah and colleagues, exhibits abnormal pathological and behavioral phenotypes that resemble PD. These mice display nonfibrillar α-synuclein-positive inclusions as opposed to fibrillar ones in PD, loss of dopaminergic synapses, and a motor deficit (83). In another model, expression of either wild type or A53T in mice showed perikaryal and neuritic accumulations of α-synuclein, neuromuscular degeneration, and reduced rotor rod performance in both animals (128). On the other hand, some studies showed the mutant-specific pathological and behavioral phenotypes. Giasson *et al.* reported that mice expressing the A53T mutant, but not wild type, developed neuronal cytoplasmic LB-like inclusion bodies, axonal degeneration in motor neurons, and severe motor impairment (39). Mice generated by M.K. Lee *et al.* showed that overexpression of the A53T mutant, but not the wild type or A30P, causes α-synuclein aggregation and motor abnormality (76). On the contrary, Neumann *et al.* showed a drastic accumulation of proteinase K-resistant, hyperphosphorylated α-synuclein and progressive locomotor deficits in A30P transgenic mice (90). In addition to the transgenic mice models, viral vector-mediated overexpression of wild type or mutant forms of α-synuclein produced selective dopaminergic neuron loss and synucleinopathy lesions (65, 79). Although none of these models seems to recapitulate perfectly the clinical and pathological features of PD, each model provides tools to investigate the fundamental roles of α-synuclein in certain aspects of pathogenesis.

Although the mechanism by which α-synuclein is involved in PD pathogenesis remains elusive, the following lines of evidence support the hypothesis that α-synuclein causes neurodegeneration through a "gain-of-toxic-function" mechanism: (a) The mutations in the α-synuclein gene that are linked to rare cases of familial parkinsonism show an autosomal dominant, rather than recessive, inheritance (69, 101). (b) The pathological features of PD, dopaminergic neuronal loss and LB formation, can be replicated by simply overexpressing α-synuclein in transgenic fly (5, 29). The finding that drosophila does not have an α-synuclein homologue (32, 106) rules out a possible dominant-negative effect of transgene-derived proteins. And, as mentioned above, some transgenic mice overexpressing wild-type or mutant α-synuclein also show inclusion bodies and neurodegenerative phenotypes. (c) The lack of a neurodegenerative phenotype in knockout mice suggests that neurodegeneration is not a "loss-of-function" phenotype of α-synuclein (1, 13). These observations are consistent with the notion that α-synuclein exerts its role in PD pathogenesis by acquiring a toxic function rather than a loss of normal function. The fact that the insoluble inclusion bodies of α-synuclein are associated with neurodegeneration in both human and transgenic animal models prompted the hypothesis that α-synuclein gains its toxic function through the aggregation process.

α-SYNUCLEIN AGGREGATION PROCESS

In vitro aggregation process

Purified recombinant α-synuclein can spontaneously form fibrillar aggregates that show all classical properties of amyloid fibrils, such as cross β-structure in x-ray diffraction, typical electron microscopic morphology, and green birefringence by Congo red (18, 110). Like other amyloidogenic aggregation, fibrillation of α-synuclein is a nucleation-dependent process (134).

Various spectrophotometric and hydrodynamic analyses showed that in dilute solution, both wild-type and mutant monomeric α-synuclein possess characteristics of unfolded proteins (125, 132). However, small-angle x-ray scattering analysis suggests that the "natively unfolded" conformation of α-synuclein is more compact than a perfect random coil conformation (125). Consistent with this observation, some residual helical structure in the N-terminus of the protein has been detected by high-resolution characterization of conformational propensities using solution NMR spectroscopy (12). Interestingly, a region of this residual structure is disrupted by the A30P mutation, whereas the A53T mutant maintains the helical preference and shows slightly enhanced regional preference for β-sheet-like conformations around the mutation site (12).

Fibrillation of α-synuclein is initiated by the acquisition of a partially folded conformation (125), which is subsequently stabilized by self-association (124). Prior to the formation of fibril, several nonfibrillar oligomeric aggregates, or protofibrils, have been identified (40) (Fig. 1). These protofibrils are enriched with β-sheet motifs, suggesting that they may be structurally related to fibrils (130). *In vitro* studies showed that fibril formation is accompanied by a reduction in the level of protofibrils (17, 24), supporting the notion that the protofibrils are the on-pathway intermediates of the fibrillation process. The earliest and most common protofibrillar species are spherical with an average height of 4.2 nm (24). The spherical oligomers are thought to undergo head-to-tail associations to form elongated chain- (18) and ring-like (24) protofibrillar species.

In their search for a potential pathogenic property of α-synuclein protofibrils, Lansbury and colleagues demonstrated that only protofibrillar α-synuclein, but not monomers or fibrils, binds tightly to and permeabilizes synthetic lipid vesicles (130). The protofibril-mediated membrane permeabilization occurs preferentially for low-molecular-mass molecules, suggesting a pore-like mechanism (129). Furthermore, recent

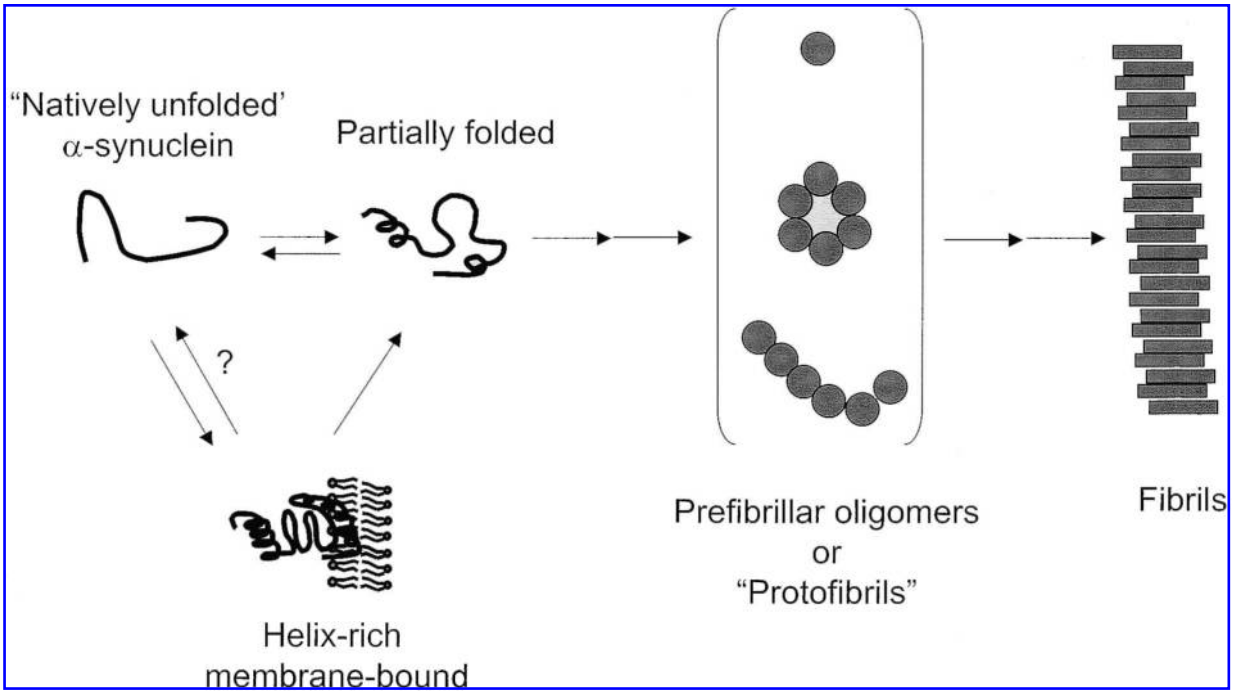

FIG. 1. α-Synuclein conformers in the fibrillation process. The native state of α-synuclein in solution is characterized by unfolded conformation. A partially folded conformation can be induced upon exposure to misfolding stresses and stabilized by self-associations to form dimers and oligomers. The oligomers with partial conformation gain high-density β-structure, as they become larger prefibrillar oligomers, or protofibrils. Protofibrils are believed to be "on-pathway" intermediates of fibrillation based on their conformational properties and kinetic behavior. The native state of membrane-bound α-synuclein is enriched with α-helical structure and can also undergo the aggregation process in the membranes. However, it remains unknown whether the binding of α-synuclein to the membrane is reversible or what controls the steady-state distribution of α-synuclein between membrane compartments and cytosol.

findings show that the two pathogenic mutations (A53T and A30P) promote the formation of annular pore-like protofibrils and result in an increased permeabilization activity relative to the wild-type protein (24, 71, 129). These results support the hypothesis that the intermediates (protofibrils), rather than the final product (fibrils) of the fibrillation process, are the pathogenic aggregate species that are responsible for neuronal cell death in PD. Although this idea needs to be tested in biological systems, a recent study provides a biochemical basis for the importance of protofibrils in dopaminergic neuron-selective degeneration (19). In this study, Conway *et al.* screened a compound library for fibrillation inhibitors and initially identified 15 compounds, 14 of which were catecholamines, including dopamine and its precursor L-Dopa. Further analysis showed that an oxidized metabolite of dopamine, dopamine quinone, stabilizes protofibrils by forming a dopamine–α-synuclein adduct, which slows the transition from protofibril to fibril. This study suggests that the stabilization of protofibrils may lead to the selective dopaminergic degeneration in PD, thus offering a mechanistic link between three well established pathological features of PD: oxidative stress, dopaminergic neuronal loss, and α-synuclein aggregation.

Factors influencing in vivo aggregation

In addition to the intrinsic conformational properties of individual proteins, there are several other considerations that influence protein folding and aggregation in cells.

Molecular crowding. Unlike the dilute solutions that are usually used in test-tube studies, the cytosolic environment is highly "crowded" with other macromolecules. This molecular crowding has significant quantitative effects on the rates and the equilibria of macromolecular interactions (27, 87). In fact, experimental evidence shows that the addition of crowding agents enhances both the physiological self-association of monomeric subunits (105) and the nonproductive aggregation of partially folded proteins (127). Addition of crowding agents also accelerates both α-synuclein protofibril formation and protofibril-to-fibril transition (116, 126). Therefore, the *in vivo* α-synuclein aggregation process is likely to be under the influence of the excluded volume effect of crowded cytosol.

Chemical modifications. Cellular proteins are subjected to various chemical modifications, such as phosphorylation, glycosylation, and oxidative modification of amino acid side chains. These modifications are likely to have direct effects on the normal conformation of the protein, thus affecting the aggregation propensity of the protein. α-Synuclein can be phosphorylated on multiple serine and tyrosine residues by several protein kinases in cultured cells and *in vitro* (26, 88, 89, 93, 102). In an effort to address the effects of phosphorylation on the aggregation of α-synuclein, Iwatsubo and colleagues showed that phosphorylation at Ser^{129} promoted fibrillation *in vitro,* and this residue was extensively phosphorylated in human synucleinopathy lesions (33). Oxidative and nitrative modifications of α-synuclein also affect the fibrillation process both *in vitro* and in cells (44, 97, 118), and nitrated α-synuclein has been observed in synucleinopathy lesions (37).

Molecular interactions. Cellular proteins are often engaged in physical interactions with other molecules, ranging from small molecules to lipid membranes. These interactions normally result in alterations in protein conformation and may change the aggregation propensity of the protein (8). A small fraction of cellular α-synuclein is associated with membranous compartments in the brain (36, 50, 61, 73). *In vitro,* recombinant α-synuclein binds to the negatively charged surface of phospholipid liposomes (22, 57, 130). Upon binding to liposomes, α-synuclein shows a dramatic increase in helical content (22, 25). When aggregation rates are compared, brain membrane-bound α-synuclein shows a higher propensity for aggregation than the cytosolic form. In addition, the aggregates formed in the membranes were capable of seeding aggregation of the cytosolic form (73). Interactions of α-synuclein with polyunsaturated fatty acids and cytoplasmic triglyceride droplets also appear to induce oligomerization of the protein (16, 100). In addition to lipids, α-synuclein interacts with several proteins (2, 3, 28, 54–56, 94, 98). Some of these proteins, such as tubulin and synphilin-1, seem to promote α-synuclein aggregation (3, 14).

Molecular chaperones. Molecular chaperones assist the folding of newly synthesized polypeptides and refolding of misfolded proteins, which otherwise tend to self-associate to form protein aggregates that often irreversibly lock the nonnative conformation (43). These folding catalyst proteins are particularly needed in the crowded environment in which the association constants for self-assembly of misfolded proteins are significantly increased. Interestingly, macromolecular crowding can also enhance the effectiveness of molecular chaperones in assisting proper folding and preventing aggregation (82, 127). Indeed, a recent study shows that overexpression of torsin A and the heat-shock proteins inhibits the formation of α-synuclein-positive inclusions (85). Furthermore, in a fruit fly model, hsp70 family chaperones alleviate neurodegenerative phenotypes caused by overexpression of α-synuclein (5), suggesting that molecular chaperones may play a role in preventing proteins from transforming into the pathogenic conformation.

Protein degradation. Proteins that fail to fold correctly are targeted for degradation systems, such as the ubiquitin-proteasome pathway and autophagy. Failure in these systems leads to the accumulation of misfolded proteins, which in turn results in protein aggregation. It is therefore noteworthy that two other genetic components that underlie the pathogenesis of familial PD are components of the protein degradation system: parkin and ubiquitin C-terminal hydrolase (UCH)-L1 (66, 77, 80). Parkin is an E3 ubiquitin ligase (48, 49, 114, 136), an enzyme that catalyzes the conjugation of ubiquitin to proteins that are to be degraded by the 26S proteasome complex (133). Recent evidence suggests that parkin might be involved in the metabolism and aggregation of α-synuclein (14, 115). UCH-L1 has been known to hydrolyze isopeptide bonds within the multiubiquitin chains to recycle the monomeric ubiquitin (70). In addition, a recent study showed that this protein also has a ligase activity that ubiquitinates α-synuclein–ubiquitin conjugates to produce polyubiquitinated proteins (78). The same

study also showed that the ligase activity of UCH-L1 forms polyubiquitin chains through Lys^{63}, instead of Lys^{48}, the ubiquitin acceptor residue that is involved in the proteasome degradation. Therefore, overexpression of UCH-L1 promotes the formation of nondegradable polyubiquitin conjugates of α-synuclein, leading to the accumulation of α-synuclein (78). Importantly, the PD-linked I93M mutant form of UCH-L1 has higher ligase activity than wild type, and this activity of I93M is inhibited by the S18Y variant (78), which was linked to a decreased susceptibility to PD.

Identification of mutations in these genes in familial PD patients raised the possibility that abnormal protein degradation due to failures in the ubiquitin-proteasome pathway may be the cause of PD. However, the role of the ubiquitin-proteasome pathway in α-synuclein degradation remains controversial. Some studies show that the ubiquitin-proteasome pathway is responsible for α-synuclein degradation (9, 86), and that inhibition of this pathway results in aggregation (84, 86, 104). However, other studies failed to find the effect of selective proteasome inhibitors on α-synuclein metabolism and aggregation (4, 97). In addition, there is also a report that α-synuclein is degraded by proteasome without undergoing ubiquitination (122).

Cellular aggregation process

Protein aggregation in cells often yields an end product of large intracellular foci termed inclusion bodies. Considering the high tendency of misfolded proteins to self-associate, and the reduced diffusion rate in the crowded cytoplasmic environment, one might predict multiple inclusion bodies in a single cell. In contrast to this prediction, cytoplasmic inclusion bodies in postmortem brains or in cultured mammalian cells are present in low number, usually only one per cell. Recent evidence shows that aggregation of proteins and formation of inclusion bodies are separate processes that are coordinated by the cytoplasmic transport apparatus (35, 58, 59). Aggregation of proteins occurs throughout the cytoplasm, resulting in a number of small aggregate particles. These particles are deposited in the pericentriolar region, adjacent to the microtubule organizing center, by retrograde transport on microtubules. These microtubule-dependent inclusion bodies are called aggresomes (58). The aggresome model explains that inclusion bodies are deposits of numerous individual protein aggregates.

LBs contain fibrillar aggregates of α-synuclein, implying that the fibrillation process *in vivo* is somehow integrated into the inclusion-forming process. In an attempt to understand α-synuclein fibrillation in the context of the inclusion-forming process, our group has established a cell system in which overexpression of α-synuclein results in inclusion bodies (72). In these cells, small punctate aggregates appear at early time points throughout the cytoplasm, followed by large pericentriolar inclusion bodies. Treating the cells with nocodazole, a microtubule-disrupting agent, results in the reduction in the number of inclusions and a concomitant increase in the number of small aggregate particles, suggesting that α-synuclein inclusions are formed by the aggresome mechanism. Using ultrastructural characterization and fibril-specific dye-binding analysis, we have demonstrated that the small aggregates are nonfibrillar spherical aggregates and that the inclusion bodies are filled with fibrillar aggregates that resemble the ones found in the LBs (72). These results suggest that *in vivo,* α-synuclein fibrillation is similar to the *in vitro* process, in which protofibrillar intermediates are involved in the fibrillation. Interestingly, nocodazole treatment leads to the accumulation of unusually large peripheral aggregates, presumably because of the inability to transport the small aggregates to the inclusion-forming site, which allows them to grow at the initial sites. Despite their large size, these peripheral aggregates maintained the characteristics of spherical protofibrils (72), suggesting that in cells, the protofibril-to-fibril transition is not a diffusion-driven process and requires microtubule-dependent deposition of protofibrils in the pericentriolar region (Fig. 2). This study clearly demonstrates that *in vivo,* α-synuclein fibrillation is tightly linked to the microtubule-dependent inclusion-forming process.

MITOCHONDRIAL DEFECTS AND α-SYNUCLEIN AGGREGATION

The most notable outcomes of mitochondrial dysfunction with regard to the protein folding and aggregation are the increase of free radical generation and the reduction in ATP production. Free radicals cause covalent modifications of amino acid residues, which subsequently destabilize the native conformation of proteins (23). Hydroxyl radicals produced by iron-catalyzed oxidation promote fibrillation of α-synuclein *in vitro* (44). Oxidative reactions from iron and free radical generators, such as dopamine and hydrogen peroxide, also stimulate the aggregation of α-synuclein in human neuroblastoma cells (95). In an independent set of studies, Ischiropoulos and colleagues showed that incubation of recombinant α-synuclein with nitrating agents stimulates nitrative modification and oligomerization of the protein (118). The oligomers and fibrils so formed were stabilized by intermolecular dityrosine cross-linking via oxidation of tyrosine. The same group confirmed the role of nitrative modification of α-synuclein in the aggregation in HEK293 cells stably expressing α-synuclein. Exposure of the cells to nitrating agents resulted in nitration of α-synuclein and the formation of α-synuclein-positive perinuclear inclusion bodies (97). Furthermore, nitration on the tyrosine residues of α-synuclein was found in all synucleinopathy lesions including LBs of PD and dementia with Lewy bodies and glial inclusion bodies of multiple-system atrophy (37), suggesting that oxidative and nitrative stresses are involved in the mechanisms underlying α-synuclein aggregation in the pathogenic process. Collectively, these studies support the hypothesis that increased generation of oxidative and nitrative radicals, often caused by mitochondrial dysfunction, results in covalent modification of α-synuclein, leading to conformational change and aggregation.

Considering the fact that ATP is required for the proper function of molecular chaperones, the ubiquitin-proteasome system, and the autophagic-lysosomal pathway (63, 113), it is predicted that a reduction of ATP production due to the mitochondrial defects would have a significant impact on the accumulation and aggregation of misfolded proteins. Indeed, some studies have shown a correlation between ATP deple-

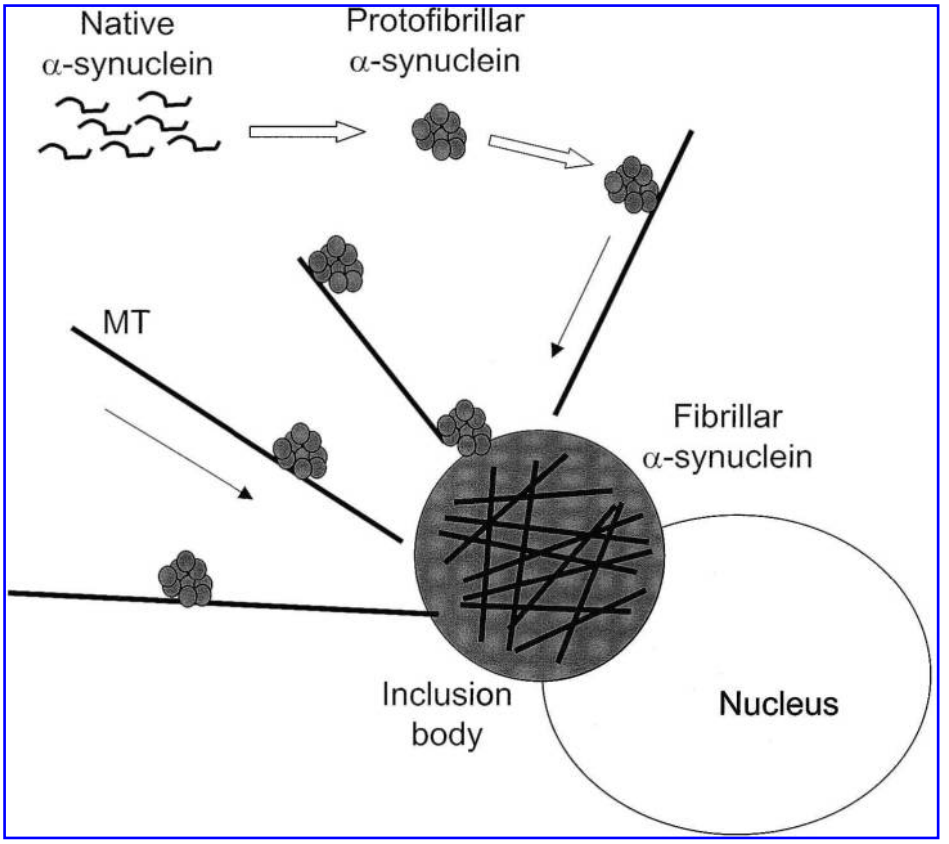

FIG. 2. Working model of α-synuclein fibrillation in cells. Small protofibrillar aggregates formed throughout the cytoplasm are transported into the pericentriolar region in a microtubule (MT)-dependent manner. Protofibril-to-fibril transition seems to occur after the protofibrils are accumulated in the pericentriolar region. See text for the details.

tion and cellular protein aggregation in cultured cells (34, 91). It seems reasonable that protein misfolding and aggregation can be induced by mitochondrial dysfunction due to the combined effects of increased levels of free radicals and decreased levels of ATP, with the former elevating the rate of protein misfolding and the latter reducing the ability to remove the misfolded proteins.

Direct evidence that mitochondrial dysfunction is responsible for α-synuclein aggregation and neurodegeneration was provided in a recent study by Greenamyre and colleagues (11). They showed that systemic administration of a complex I inhibitor, rotenone, to rats results in major clinical and pathological signs of human PD, including α-synuclein-positive inclusion bodies, selective dopaminergic loss in the substantia nigra, and reduced motor skills. This study supports the hypothesis that mitochondrial defects, especially complex I impairment, are sufficient to trigger the entire pathogenic process that includes α-synuclein aggregation and neurodegeneration Similarly, in human neuroblastoma cells, chronic rotenone exposure (5 n*M*) increased α-synuclein protein levels in a week, and longer treatment (2–4 weeks) of rotenone induced oxidative damage and apoptotic cell death (112). Although this study showed an increase of detergent-insoluble α-synuclein after 4 weeks of rotenone treatment, whether this treatment indeed promoted α-synuclein aggregation was not clearly demonstrated.

Rotenone-induced α-synuclein aggregation was characterized in more detail in COS-7 cells using a higher dose of rotenone (100 n*M*) (74). In this study, rotenone treatment induced ubiquitin- and α-synuclein-positive fibrillar inclusion bodies within 3 days, whereas spontaneous aggregation was minimal at the same expression level. Small spherical α-synuclein aggregates formed throughout the cytoplasm before the appearance of inclusion bodies, and these inclusion bodies formed in the pericentriolar region, suggesting that the aggresome mechanism underlies the rotenone-induced inclusion formation. Interestingly, when rotenone was washed out with fresh medium, the small prefibrillar aggregates were progressively degraded, whereas the fibrillar inclusion bodies were relatively spared (74). The clearance of the aggregates paralleled the recovery of cellular ATP production. These results suggest that cells have the ability to protect themselves by degrading toxic aggregates, and this degradation activity may require mitochondrial energy production.

The accumulation of α-synuclein aggregates in cells is determined by a dynamic equilibrium between production and removal. The studies summarized here suggest that defects in mitochondrial function lead to an increase in α-synuclein aggregate production and a decrease in the ability to remove them, subsequently leading to the accumulation of the aggregates in the cytoplasm. Given the importance of mitochondrial function in α-synuclein aggregation dynamics, it is noteworthy that overproduction of α-synuclein results in mitochondrial dysfunction and increased levels of free radicals (47). Although the role of α-synuclein aggregation in causing mitochondrial

dysfunction was not clearly addressed in this study, the authors did show the formation of α-synuclein aggregates in their cell system. Furthermore, an *in vitro* study by Ding *et al.* showed that protofibrillar α-synuclein with membrane-permeabilizing activity binds to rat brain mitochondria much more avidly than the monomer (24). Although more studies are needed, there might be a positive feedback loop between mitochondrial defects and α-synuclein aggregation.

Although the studies described here effectively support the principle that impairment of mitochondrial respiratory chain function could lead to α-synuclein aggregation, the *in vitro* studies with acute complex I inhibition should be interpreted with caution. The most salient concern comes from the fact that the superoxide production from mitochondria requires ~90% inhibition of complex I in a preparation of brain mitochondria (131), which is much higher than the 30–40% inhibition found in PD (53, 81, 108). Also, in the rat rotenone model, pathologic and behavioral PD symptoms can be produced without significantly impairing respiration, thus without substantially reducing ATP production (11). Therefore, the acute *in vitro* model does not perfectly reflect the pathogenic cascade that links mitochondrial defects to α-synuclein aggregation and neurodegeneration. It is possible that the cumulative effect of the mild impairment of mitochondrial function over time may eventually cause increased production of free radicals and reduced production of ATP. It is also possible that there are additional components in cells, such as dopamine and iron, that can amplify the effects of minor changes in superoxide and ATP production (19, 95). Exposure to certain environmental toxins may also have synergistic effects with the subphenotypic deficits of mitochondrial function.

ARE α-SYNUCLEIN AGGREGATES CYTOTOXIC?

The cytotoxic effect of α-synuclein after the exogenous overexpression of the wild-type or mutant forms of α-synuclein has been investigated by several groups. These studies generated a wide spectrum of outcomes: some reported cytotoxic effects for all α-synuclein variants (52, 94, 107, 135, 138), whereas others found toxicity either only with the mutant forms (75, 137) or only in the presence of additional stress (60, 62, 67, 137). Some of these studies showed dopaminergic neuron-selective neurotoxicity with α-synuclein expression (135, 137, 138). Furthermore, overexpression of wild-type, but not mutant, α-synuclein showed protective effects against cytotoxic insults (21, 45, 75). One of the most important issues that may be relevant to these rather confusing results is whether the aggregation of α-synuclein is responsible for the toxicity of this protein.

In a recent study, we have shown that in COS-7 cells, cytotoxicity of α-synuclein correlated with the amount of aggregates, whereas an increase in monomer level did not affect cell viability (41). In addition, α-synuclein aggregation was also associated with fragmentation of the Golgi apparatus and impairment of protein trafficking through the biosynthetic pathway (41). Although it is not clear as yet whether Golgi fragmentation is linked to cell death, these results support the notion that α-synuclein gains pathogenic function through forming higher order quaternary structures to damage specific cellular targets. Interestingly, the same study showed that both cell death and Golgi fragmentation were tightly correlated with the production of prefibrillar aggregates, but not with the formation of fibrillar inclusions, suggesting that the prefibrillar aggregates might be responsible for the cellular impairment. Although this study shows a strong correlation between α-synuclein aggregation and cellular impairment, it is still not clear whether the aggregates themselves are toxic agents or whether the *process* of aggregation is actually responsible for the impairment.

As discussed above, normal mitochondrial function is thought to be important in keeping the cytoplasm free of α-synuclein aggregates by preventing misfolding/aggregation and by clearing of the preformed aggregates. With the recent evidence that certain aggregate forms (protofibril or other species) of α-synuclein rather than the monomers are associated with cell death and other functional impairments, it is speculated that α-synuclein aggregate formation is one of the routes through which mitochondrial defects lead to neurodegeneration

CONCLUDING REMARKS

The fibrillation process of α-synuclein involves a series of conformational changes and several metastable prefibrillar intermediates both *in vitro* and *in vivo*. Mitochondrial dysfunction leads to cytoplasmic accumulation of α-synuclein aggregates by promoting the production of aggregates and by inactivating cellular mechanisms to remove the preformed aggregates. In addition, studies in cultured cells and animals show that the accumulation of α-synuclein aggregates, not the monomeric α-synuclein, is associated with cellular impairments and death. These findings suggest that α-synuclein aggregation might represent one of the principal mechanisms underlying the pathogenesis of PD and other LB diseases. However, the causative role of α-synuclein aggregates in neurodegeneration still remains speculative. The critical questions include whether, and to what extent, α-synuclein aggregation contributes to neuronal loss and the progress of LB diseases, and whether α-synuclein aggregation is the critical link that connects mitochondrial defects to neurodegeneration. One way to approach these problems is to develop genetic and chemical tools to manipulate the aggregation process. Recent progress in cell and animal models should provide useful systems for the screening of various genes and chemical compounds in the search for aggregation modulators.

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ABBREVIATIONS

LB, Lewy body; PD, Parkinson's disease; UCH, ubiquitin C-terminal hydrolase.

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